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The composition and metabolism of Bracken (Pteris Aquilina), with special reference to its influence on animal health.

b y

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Preface

The first cases of bracken poisoning were recorded in 1893 by Penberthy and Storrar. Since then, most investigators have come to the conclusion that the fatal results produced by feeding cattle and horses on a diet composed of bracken, are due to the presence of a cumulative poison. No conclusive evidence of bracken poisoning in sheep has been recorded.

During recent years there has been a heavy mortality of cattle grazing on bracken infested hill land in Scotland. It has been suggested that this might be due to the Hill Cattle Subsidy Scheme (1946) requiring the cattle to be on the hill continuously for 16 weeks. Although bracken poisoning has caused much anxiety to farmers in Great Britain, it has also been a source of trouble in other parts of the world, e.g. Europe, the United States of America, and Australia.

In spite of the work of several investigators under different experimental conditions, the aetiology remains obscure and as yet, no complete examination of bracken for toxic constituents has been made. Since Greshoff in 1908 mentioned the presence in young bracken shoots of a glucoside, which by enzyme hydrolysis

produced hydrocyanic acid, little attention has been paid to this constituent. It was therefore considered essential that an examination should be made of the composition and metabolism of bracken including an examination of the significance of this cyanide fraction. In the last three or four years studies on the inactivation of thiamine by bracken have been reported by different investigators in Great Britain and the United States of America. Since these experiments have been confined to rats and horses, the opportunity has been taken to extend this work and examine the significance of the antithiamine activity of bracken in the aetiology of bracken poisoning in ruminant animals.

P A R T I

Review of Literature of Bracken Poisoning.

Review of Literature of Bracken Poisoning.

A. Historical.

The toxicity of bracken has been the subject of much discussion since it was first recorded in 1893 by Penberthy and Storrar (25),(33), but sufficient experimental evidence has since been obtained to confirm that bracken, either fresh or dry, may be poisonous.

Penberthy (25) noticed that the majority of the cases occurred sometime after rain, following a drought of severe intensity, and that the disease was specifically confined to bovines and did not occur in horses, pigs and sheep under the same environmental conditions. Serious losses of cattle in Surrey, Kent, Lincolnshire, the West of England, the Midlands and Ireland, were reported by Penberthy.

In the same year, a similar outbreak in cattle was mentioned by Storrar (33). In this case an examination of the pasture on which the cattle grazed showed that the young fronds of bracken had been eaten off. Although the cattle had been transferred to an aftermath, half of them died from bracken poisoning. These reported cases covered animals of

both sexes, young and old, ranging from nine months to five years of age.

In 1893 Freeman (11) described a peculiar disease in cattle in Ireland, which was not attributed to bracken. The incidence was confined to the mountain districts where the grasses were coarse but of good feeding value and there was no indication that bracken might be involved. The similarity of the symptoms to those observed by Penberthy and Storrar, however, led to the reporting of these cases.

The editorial columns of the Journal of Comparative Pathology and Therapeutics in 1894 (1), reviewing the question of poisoning by bracken, referred to the outbreak of the previous year observed by Penberthy and the suspected cases reported by Freeman in Ireland. It was pointed out that during unusual drought periods, as experienced in 1893, the cattle were compelled to eat bracken which was practically the only herbage available in many pastures, and later they showed symptoms of the disease.

Although negative results were recorded by Penberthy in a feeding trial with a young bullock fed on bracken, yet in the editorial article referred to above it is stated that the disease had been reproduced by feeding animals on turnips and bracken chopped up with the straw, the straw being gradually withdrawn. This feeding was soon

followed by the death of some of the animals and the serious illness of others. The results of this and other experiments are a sufficient indication that bracken must be regarded as a dangerous plant to cattle. Nevertheless the cause of the toxicity of the plant has remained the subject of much discussion.

Stockman (29, 30) in his 1909 and 1910 reports to the Board of Agriculture confirmed the existence of the "mysterious disease" in cattle and indicated that the fatalities were mostly in late summer and autumn. All cases were attributed to the ingestion of bracken as this was found to be available to the animals and was noticed in the areas in which the cases were reported.

McGowan (22) came to the conclusion that the clinical syndrome was not due to a poison in bracken but to the Bacillus of haemorrhagic septicaemia, which he claimed to have isolated from the blood of cattle which had died of bracken poisoning. Stockman (31, 32), Bosshart and Hagan (4), Hagan (16,17), and Kerdiles (18) criticised this view, believing the organisms isolated by McGowan to be normal post-mortem invaders and not the cause of death. In recent examinations of animals which had died from bracken poisoning, Stamp (28) found no evidence of such organisms in the blood or bone marrow.

Later in 1917 Stockman (31) conclusively proved for the first time by experimental feeding that the fronds of bracken were poisonous to cattle particularly between August and November. He fed a heifer for 9 days on a total quantity of 56 lb of bracken with negative results. Another heifer which received 30 lb of bracken in the first two days, followed by 6 lb per day for the next 5 days (total 60 lb) also gave negative results apart from slight indigestion. He next fed a bull-calf 8 months of age at the rate of 10 lb of bracken per day with other food. After a total consumption of 260 lb during a period of 26 days the symptoms began to appear and the animal died on the 30th day with typical symptoms and post-mortem findings as found in field cases. Since the symptoms and lesions observed were very similar to those described in "acute scurvy" in other species of animals, Stockman (32) investigated the possibility of avitaminosis in the presence of bracken. The experimental feeding of a 4 months old bull-calf began with a ration of 4 lb, increased after 3 days to 6 lb, of newly cut green bracken, mixed with bran and oats to eliminate the possibility of starvation. As soon as the symptoms appeared in the animal he fed a generous supply of swedes since this was known to be rich in anti-scorbutic vitamin. In spite of

looking a little brighter after eating swedes for 1 to 2 days, the animal died on the 30th day, with the usual symptoms and post-mortem lesions of bracken poisoning. A similar experiment was carried out with a 3½ month old calf with a good supply of swedes from the very beginning of the experiment. After consuming 112 lb of green bracken in 28 days the animal died on the 30th day.

Craig and Kehoe (6), reviewed the question of bracken poisoning when they affirmed that Stockman's conclusions were correct and that the disease could be produced experimentally by feeding considerable quantities of bracken fronds over a long period.

Bracken poisoning in cattle has been reported from many different parts of the world. Bosshart and Hagan (4) reported many cases during 1917, 1918 and 1919 in various places in New York State. The disease was mostly found on hilly and well drained areas of good pasture. Though the greatest susceptibility was observed in yearlings and 2 year olds, the disease was found in all ages of cattle.

Kerdiles (18) stated that quite an alarming loss of cattle from bracken poisoning occurred in Brittany. In France, Lominet and Lavielle (19) described the symptoms in a small herd of cattle fed on a diet of 75% bracken fronds. They suggested it to be a case

of bracken poisoning but this was not supported by sufficient evidence.

Losses in Tasmania have also been reported although the assumption that bracken was the responsible factor has since been questioned (2).

The apparent poisoning of sheep by bracken fern in Auckland was recorded in the New Zealand Journal of Agriculture Vol. I page 215. Though no symptoms appeared, 12 out of 70 ewes and 18 out of 300 lambs died without struggle. It was concluded from the post-mortem finding of fern shoots in a semi-digested state in the rumen, that deaths were due to the ingestion of bracken found in the grazing area. This seemed to be the first report of such a disease to occur in sheep. In this country, sheep had never been reported to be poisoned by bracken.

The illness of horses in Germany after prolonged feeding of bracken silage was recorded by Muller in 1897 (24) without reference to the proportion of bracken in the diet. Maksimoff in Russia (21), however noticed that silage prepared from the fern (pteris aquilina) and kept 9 months in ordinary pits had no detrimental effects but proved a satisfactory fodder and was consumed readily by cattle. Watson (36) also made young bracken into silage and found it harmless when fed to stock.

During January and February, 1916 Hadwin reported many deaths of horses along the Pacific Coast of North America where bracken was extremely common, particularly in Oregon and Washington and from British Columbia to Alaska. The symptoms before death, led to the condition being termed "Staggers". Hadwin (13) reported some preliminary experiments in which hay containing dried bracken was fed to an animal which died. The same hay from which the bracken had been removed was fed to a control animal which did not take the disease. Hadwin and Bruce (14) reported another experiment with 5 horses in British Columbia in which 6 lb of dried bracken added to the daily diet killed a horse in one month. Though the natural occurrence of the disease was largely confined to severe hard winters, it could also be produced experimentally in summer. The only reasons for its occurrence in winter might be that the animals got least exercise and were of lower vitality during winter. In a further paper Hadwin and Bruce (15) stated that the disease popularly known as "staggers" was due to the ingestion of dried bracken over a certain period of time. From an experiment with 4 horses it was concluded that an average of about 6 lb of bracken had to be included in the daily diet to kill a horse in one month. Though there was no

reference to any such disease in horses in Great Britain, the authors stated that some cases in the South of England had been noted, after being fed on ferny hay from Northumberland.

Since 1935 a number of outbreaks of bracken poisoning have been reported and some investigations of the problem have been made, particularly in the last four or five years. Lynch (20) noted the disease in heifers and bullocks in 1935 and on enquiry it was discovered that bracken was used as litter, often in the fresh condition, and the animals ate it. Although it has generally been believed that the young bracken fronds are responsible for the poisoning in cattle, Craig and Davies (5) in 1940, recorded cases of poisoning in October and this led them to assert that old and not young bracken was responsible. In 1944 Fletcher (10) in a clinical communication distinguished two types of poisoning:-

1 - The enteritic

2 - Acute laryngeal.

He considered the old and withered bracken to be most toxic, yet cases might arise in early summer due to the increased toxicity of young shoots when frosted or to the cutting of bracken for eradication purposes leading to withering and increased toxicity. In June 1944 he reported the case of a Black-face ewe with

typical post-mortem symptoms of bracken poisoning though he admitted that evidence of the occurrence of the disease in sheep was incomplete and needed thorough investigation. Shearer (27) in 1945 claimed that a fatal result was produced in a heifer by feeding a diet composed solely of bracken for 74 days. The symptoms and post-mortem lesions described by former workers were observed. Another heifer whose diet was composed of equal parts of bracken and normal food, ate three quarters of the amount of bracken which proved fatal to the other animal, over a similar period of time, but showed no harmful effect. He concluded that:-

1. Bracken poisoning occurs only when consumed in large quantities for a considerable period.
2. Irritation of the gut causes increased permeability of the gut wall through which toxins are more readily absorbed.
3. The poison in bracken is not cumulative in nature since -

a. The characteristic lesions are of an acute nature and not chronic.

b. It is only thought to be cumulative because time is needed to consume large amounts of bracken.

Recently Boddie (3) has emphasised the following:-

1. There is a considerable difference of opinion as

to whether bracken itself causes the disease or whether it acts only as an exciting agent for bacterial action.

2. There is a divergency of opinion in the country; one part of the country accepts the explanation of deaths in cattle on the grounds of bracken toxicity, while the other part of the country strongly disagrees with this view.

3. Variations in the weather may have an influence on the quantity of bracken eaten or on the degree of toxicity of bracken.

Boddie also suggested that by removing the cattle from hill land to low ground free from bracken at 3 weekly intervals the losses may be avoided.

Much attention has been directed towards the anti-thiamin activity of bracken. This fact was noted by Weswig, Freed, and Haag (38) in 1946 when they fed rats on a diet containing 40% bracken and in one month caused deaths with the symptoms of thiamin deficiency. In 1949, the work of Thomas and Walker (34) confirmed these findings. Rats on a diet containing 40% bracken and providing adequate amounts of thiamin, lost weight and died within 30 days. Control groups receiving large supplementary doses of thiamin made continuous increases in body weight. They stated that the results of both feeding trials and incubation tests showed the presence in bracken of a substance

which is capable of inactivating thiamin. The nature of this substance was uncertain although it was found to be destroyed by hydrolysis with dilute hydrochloric acid and by autoclaving. Though these results obtained with rats could not be applied to cattle or sheep, yet Thomas and Walker suggested that bracken poisoning in farm livestock may be due to an induced thiamin deficiency.

Evans and Evans (7, 8) also confirmed this work on rats and mentioned the presence of a mechanism in common bracken which renders aneurin in the diet unavailable to the animal. Roberts, et. al. (26) reported the production of bracken staggers in a horse with the typical picture of avitaminosis B_1. Daily subcutaneous injections of 50 to 100 mg of aneurin, without changing the bracken diet, led to a rapid recovery of the animal. In 1950, Evans et. al. (9) claimed that in bovine bracken poisoning when clinical symptoms were evident there was also a state of B_1 deficiency which appeared to be complicated with other factors. Relatively little work on the occurrence and significance of B_1 deficiency in adult ruminant animals has, however, yet been reported.

B. Symptoms and Post-mortem appearances.

Bracken poisoning symptoms appear to be very changeable according to species, and to environmental conditions. Cases of the disease may be divided into per-acute, acute, and chronic types.

1 - Per-acute

The animal may never be noticed ill, but though appearing perfectly normal when last seen alive, is found dead a few hours later. Blood is commonly noticed coming from the nose and anus after death.

2 - Acute

These cases linger from 24 to 72 hours as a rule. The symptoms have been variously described by several investigators.

3 - Chronic

This type which was first distinguished by Bosshart and Hagan (4) lasts from 4 to 10 days or longer, and deaths almost invariably follow.

The symptoms first reported by Penberthy (25) in cattle are as follows:—
The animals die within 1 to 5 days showing extreme dullness, drooping of the head and ears, the back usually a little arched, the skin dry and hard. The eyes become more and more sunken, and usually show

some discharge whilst foaming at the mouth, blood and mucus discharging from the nostrils, and blood stained faeces, or blood clots discharging from the anus are characteristic. Constipation is often followed by diarrhoea, occasional tenesmus, and swelling of the throat and breast. The breathing is a little accelerated, the pulse 70 to 80, small and feeble. The temperature is very high (over 109°F has been noted) and food frequently refused. The depression and emaciation increase and the victims usually succumb with very little struggle though a little excitement has been noticed in some cases.

Symptoms of cases reported by Storrar (33), Freeman (11) and Stockman (31,32) were almost the same as those found by Penberthy. Freeman (11), has reported some per-acute cases, where no symptoms appeared at all until the animals were found dead with blood passing out from the anus, valva and nostrils.

In horses, nervous symptoms are typical, the animals showing uncertain gait, and loss of equilibrium as well as general unthriftiness. Although the appetite is usually good the animals are prone to constipation. The eyes become congested, the flanks tucked up, and the animal stands unsteadily with its

legs spread out and with a distinct intoxicated look; it may frequently fall. The temperature is not abnormally high in horses and no symptoms of bleeding from any part of the animal have been noticed during the course of the disease although this is a characteristic symptom of the disease in cattle.

Bosshart and Hagan (4) have distinguished two types of the disease in cattle:- 1. The acute type which is characterised by extremely high temperature (106 - 109°F) with haemorrhages, and death within 3 days; 2. the chronic type lasting from 4 to 10 days or longer with death almost invariably following.

Lynch (20) noticed a high temperature (106°F - 107°F) in the case of heifers and 4 - 8 month old bullocks but in the oldest animals of 8 months there was no abnormal temperature (102°F). The animals were noticed to be snoring, with slight salivation and were off their feed. Slight oedema in the throat region was also noticed and slight pressure intensified the respiratory sound. The deaths were quick and all animals died within one week without symptoms of bracken poisoning.

Fletcher (10) has described two types of symptom:- 1. - The enteritic type showing depression, high temperature (103 - 107°F) weak pulse, and blood clots in the faeces. This type is the more common.

2. - The laryngitic type showing swelling in the throat region, roaring and difficulty in breathing, increased respiration and a high temperature.

Shearer (27) noticed in his cases the same symptoms as reported by Penberthy and other workers.

On post-mortem examination the most characteristic lesions, which have been described by Stockman (31) Craig and Davies (5), and Watt (37) are petechial haemorrhages in the alimentary canal and internal organs particularly in the heart, lungs and muscles and under the skin. Congestion of the liver and kidneys are also found.

The most striking lesions are seen in the abdomen. The walls of the intestines are splashed with blood, and the large intestine and blind gut are often completely filled with blood clots of a bright red colour. In the fourth stomach haemorrhages and ulcers about the size of a sixpence may be found. In addition to these Stamp (28) has reported large (1 - 2 cm) sub-acute ulcers in the latter part of the small intestines and caecum, the walls and floor of the ulcers consisting of pale granulation tissue.

C. Treatment and prevention of bracken poisoning.

Regarding the treatment of cases of bracken poisoning no suggestions appear to have any real curative value, because when symptoms are first noticed the bracken has already exerted its toxic effect and the animal is past saving.

Watt (37) and Boddie (3) have pointed out that the animals must be removed to bracken-free grazing, and some authorities recommend a purgative (linseed oil or Epsom salts). The death of some animals may occur even three weeks after removing them from the affected pasture. This is attributed to the amounts of toxic principles already accumulated by the animal as well as those present in the bracken still in the rumen. The most important point is to break the chain of accumulation and give the animals a chance to eliminate the toxic substances from the body. In the cases of poisoning which occur in cattle bedded on bracken and horses fed on hay containing bracken, the bedding and hay should be changed for a time, to interrupt the accumulation chain. It has been observed that bracken poisoning occurs most frequently where large quantities of bracken are regularly consumed permitting an accumulation of toxic principle in the animal body. If this building up process is

interrupted the chances of an outbreak are correspondingly lessened.

D. Toxic principles in Bracken

The following suggestions regarding the nature of the toxic principle in bracken were made by Stockman (31):-

1. It is usually present in comparatively small amounts.
2. It belongs to the class of vegetable toxins (Phytotoxins).
3. It is cumulative in action.
4. It requires a certain time to establish its full effect, after which severe illness may begin in an explosive manner.
5. The toxic principle is the same as that present in soya meal.
6. It is fairly resistant to heat.

As Shearer (27) and Boddie (3), have stated, no chemical proof has been forthcoming regarding the possible accuracy of the suggestions made by Stockman (31). In fact, a search of the literature shows that no adequate chemical examination of bracken has been undertaken and until recently there has been little intensive work on the problem at all.

Although Greshoff (12) mentioned in 1908 the presence of a cyanogenetic glucoside in young bracken shoots, no thorough investigation was made to

study the nature and the significance of this glucoside in bracken.

A search has, however, been made by Shearer (27) for a substance to which the poisonous properties of bracken could be ascribed. Repeated attempts to reveal the presence of alkaloids, phytotoxins, saponins or glucosides by the usual methods of examination were unsuccessful and attention was then directed to the type of tannin contained in the plant. This showed that there are varying amounts of a catechol tannin but no other possibly toxic substance could be detected. Moon and Pal (23), observed an increase in the tannin content in September, but this was not comparable with the increase found by Shearer (27). An attempt was also made to elucidate the significance of tannin in the in-vitro digestion of bracken leaf protein, with unsuccessful results.

A number of agricultural research stations are now investigating the toxicity of bracken but despite the recent work on the antithiamin activity of bracken much work would appear to be necessary before the problem of bracken poisoning in ruminants is solved.

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PART II

Feeding Experiments

Feeding Experiments.

Introduction

Owing to the great lack of information on the subject of bracken poisoning a scheme of feeding experiments was planned to investigate some of the factors which might be involved.

One experiment was carried out to determine whether or not the mineral fraction of bracken could be related to the poisoning and if so, to study in some detail the metabolism of bracken ash.

Feeding experiments were also carried out with fresh bracken at different stages of growth, using young bracken in the early part of the growing season, mature bracken in the summer and autumn, and young bracken again (second growth), in the late autumn. (Fig. 1 and 2.) In these experiments the occurrence and metabolism of the cyanogenetic glucoside fraction was examined. The possible production of vitamin B_1 deficiency in the animals fed on fresh bracken was also investigated.

To study the possibility of producing poisoning with dried bracken a considerable quantity of young bracken was cut on 30th June 1949 and dried in a commercial grass drier for feeding tests at a later date. Part of this dried bracken was fed without further treatment and part was hammer milled and cubed

Fig. 1 - Young bracken.

Fig. 2 - Mature bracken.

before feeding. A further portion of the dried bracken was commercially extracted with alcohol in order to separate the cyanogenetic fraction, the extracted meal being cubed for feeding and the extract being fed separately in admixture with hay.

To complete the work on the significance of the cyanide fraction of bracken, feeding experiments were also made with amounts of sodium cyanide equivalent to the intake of HCN from the bracken consumed by those experimental animals which died from bracken poisoning.

Sources of bracken used.

In a previous pilot experiment in August 1948, a bullock fed on fresh bracken collected from the Pentland hills at Boghall Experimental Farm died with all the symptoms of bracken poisoning. As this source of bracken was not very extensive, search was made for a more suitable supply in the same vicinity. This was found a few miles further south on the lower slopes of Carnethy hill (800') where the bracken was both extensive and reasonably accessible.

Fresh bracken samples were collected from both Carnethy hill and Boghall farm in May and June, 1949 and analysis showed them to be similar in HCN content. Although the Boghall bracken had been shown to be poisonous to cattle in 1948 it was decided to carry

out the new series of experiments using only bracken from Carnethy hill in view of its similar analysis and its presence in considerable quantity on an accessible site.

Experimental animals

The animals used in the whole series of experiments were three bullocks and seven sheep. Bullock I was a black Irish beast of about 2 years of age and weighing about 8 cwt, taken from Buckholm farm, Galashiels. The other bullocks, Nos. 4 and 5, were home bred Galloway yearlings from Boghall farm, weighing about 6 cwt. The sheep used, designated B, C, D, E, F, G, and H, were all cross-bred wethers, varying in age from 1 to 2 years. The experiments carried out with these animals are shown in the following tables.

Bullock	Type of feeding	Period of Experiment.	No.of days	Results
1	A.Bracken ash + hay	30/1/49-22/3/49	52	Lived
	B.Fresh bracken (young)	18/6/49-18/7/49	30	Died
4	A.Fresh bracken (mature)	18/8/49 8/10/49	52	Lived
	B.Fresh bracken (young 2nd growth)	9/10/49 24/10/49	16	Died

Bullock	Type of feeding	Period of Experiment.	No. of days	Results
5	A. NaCN≡89.35 mg HCN per day (+hay)	15/3/50-11/4/50	28	Lived
	B. NaCN≡134.03 mg. HCN per day (+hay)	12/4/50-12/5/50	31	Lived
	C. Alcohol extract of bracken (+hay)	26/6/50-9/7/50	14	Lived

Sheep	Type of feeding	Period of Experiment.	No. of days	Result
B	Bracken ash (+ hay)	30/1/49-22/3/49	52	Lived
	A. Fresh bracken (young)	18/6/49-18/7/49	30	Lived
	B. Dried bracken	3/4/50-10/5/50	38	Lived
C	C. Alcohol extracted bracken (cubes)	11/5/50-23/6/50	44	Lived
	D. Dried bracken	24/6 - 28/6/50	5	Lived
	E. Whole bracken cubes	29/6/50-8/7/50	10	Lived
	F. Dried bracken	9/7/-31/7/50	23	Lived
D	A. Dried bracken	16/3-10/5/50	56	Lived
	B. Whole bracken cubes	11/5/50-23/6/50	44	Lived
	C. Dried bracken	24/6/50-9/7/50	16	Died
E	A. Fresh bracken (mature)	18/8/49-8/10/49	52	Lived
	B. Fresh bracken (young 2nd growth)	9/10/49-21/10/49	13	Died

Sheep	Type of feeding	Period of Experiment.	No. of days	Result
F	A. Fresh bracken (mature)	18/8/49-8/10/49	52	Lived
	B. Fresh bracken (young 2nd growth)	9/10/49-23/10/49	15	Died
G	Dried bracken	16/3/50-24/5/50	70	Died
H	NaCN≡54.108mg HCN per day (+ hay)	15/5/50-31/7/50	78	Lived

Bracken ash feeding experiment

A large quantity of mature field dried bracken was burned and the ash used for the feeding experiments. It was fed at the rate of 400 g. per day to bullock 1, and 50 g. per day to sheep C, these quantities being equivalent to 8 Kg. and 1 Kg. dried bracken respectively.

The best method found for feeding bracken ash to the bullock was to mix the 400g. ash with 1800c.c. molasses and 1000 g. chopped hay. This mixture was given in two feeds, at 10 a.m. and 5 p.m. in addition to the normal daily feed of 10 - 12 lb long hay. Any uneaten residues of the ash-molasses-hay mixture were collected each morning and incorporated with the new feed. With the sheep it was found suitable to mix 50 g. ash directly with 1000g. of chopped hay, this being given in two feeds daily. Preliminary attempts to feed the ash in the form of pellets made with molasses and dried in the oven, were unsuccessful,

and the ash-molasses-hay mixture as used for the bullock was refused by the sheep.

The feeding of bracken ash lasted for 52 days (January 30th to March 22nd 1949), but collections of urine and faeces were made only during the 1st and last 10 days of this period. Although in the 52 days the bullock received bracken ash equivalent to 416Kg. dried bracken, there was no toxic effect. The same was the case with the sheep, which received the equivalent of 52 Kg. dried bracken.

During the periods of collection, daily samples of the food fed, food residues, faeces, and urine were ashed for determination of the mineral constituents if necessary. However, as no evidence of any toxic effect due to the mineral fraction of bracken was obtained no detailed study of the mineral metabolism was considered worth while.

Fresh bracken feeding experiments

1st Experiment (young bracken)

(Bullock 1, Sheep C)

The first feeding experiment was carried out with bullock 1 and sheep C, to determine the apparent cyanide balance and its relation to the toxic effect of the bracken. The animals were fed entirely on bracken collected fresh each morning from Carnethy hill and hydrocyanic acid was determined daily in this fresh

bracken. In blood and urine samples both HCN and HCNS were determined, and the total benzoic acid excretion in the urine was also measured.

In order to determine the apparent cyanide balance the daily production of urine had to be collected quantitatively. With the bullock this was achieved by using a special rubber funnel attached to a thick rubber tube carrying the urine to an enamel basin at a low level. (Figs. 3 and 4) A little toluene was added to the urine as a preservative. In different periods of the experiment faeces were also collected by fixing a special rubber-lined canvas bag on to the rear of the bullock by means of a suitable harness (Fig. 5 and 6). Faeces were removed by unfastening a cord fixed round the bottom of the bag and this end was kept clear of the floor by means of a cord attached to the wall (Fig. 4)

The animal was tied up by the neck and prevented from turning round in its stall, but was allowed sufficient room to lie down or to make a limited forward or backward movement. This facilitated the collection of urine and faeces and minimised the scattering of food. Water was provided in a pail fixed in front of the animal whilst the food was placed in a trough at ground level with a high front to limit scattering.

Fig. 3 - Rubber funnel for collecting urine.

Fig. 4 - Collection of faeces and urine.

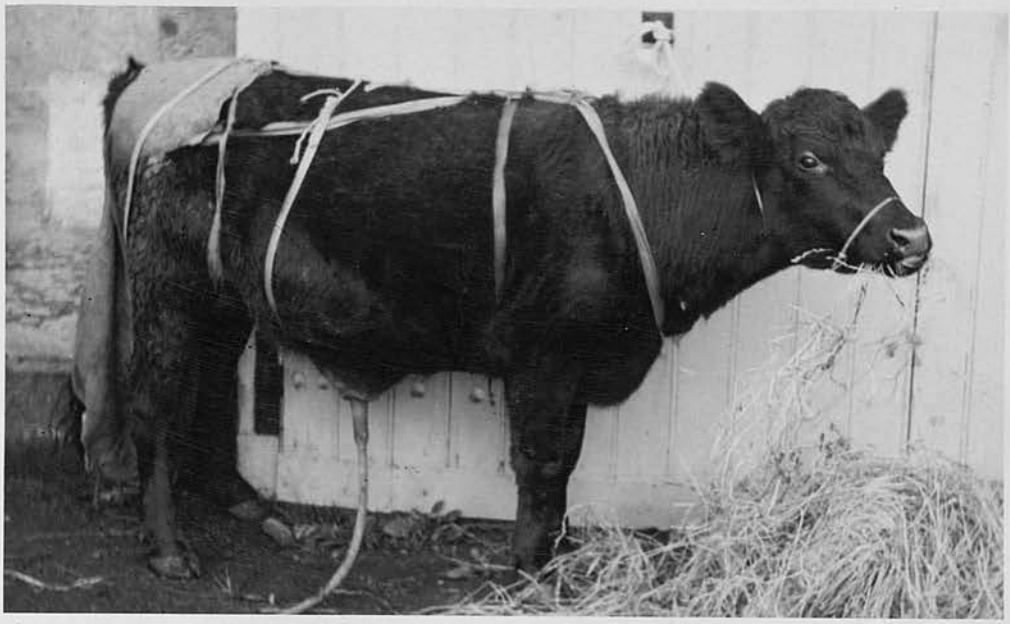


Fig. 5 - Bullock metabolic harness.

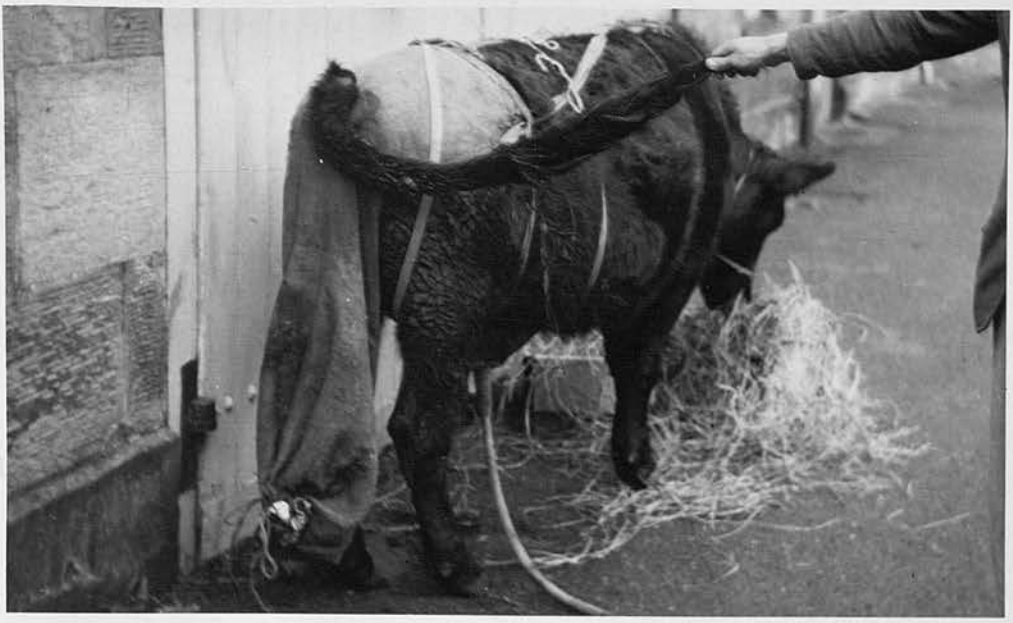


Fig. 6 - Bag for collecting faeces.

The sheep was housed in a special experimental cage (Fig. 7) with a lead-lined funnel below the wire floor to permit the collection of urine. Faeces were collected in small bags consisting of two parts as shown in Fig. 8. The two parts A and B were held together by press-studs when in use, part A being permanently fixed to the sheep by suitable harness (Fig. 9). When collecting the faeces part B was merely unbuttoned and replaced.

During the experimental period of 29 days commencing on June 18th and ending on July 16th, 1949, the bullock consumed 389 Kg. of fresh bracken with an average of 13.4Kg per day, while the sheep consumed 10.1 Kg. with a daily average of 0.35Kg. During this period the bracken cut each morning was chopped, mixed thoroughly on a clean floor, and the day's feed weighed out; approximately half of this was fed immediately, the remainder being given in the afternoon. A fresh sample was taken for analysis immediately after chopping and mixing. This sample was homogenized and used for the determination of HCN and a further sample was oven dried for moisture determination. The uneaten bracken residues were collected and weighed each morning before giving the first feed, and a sample taken for moisture determination. The total daily urine excretion was also

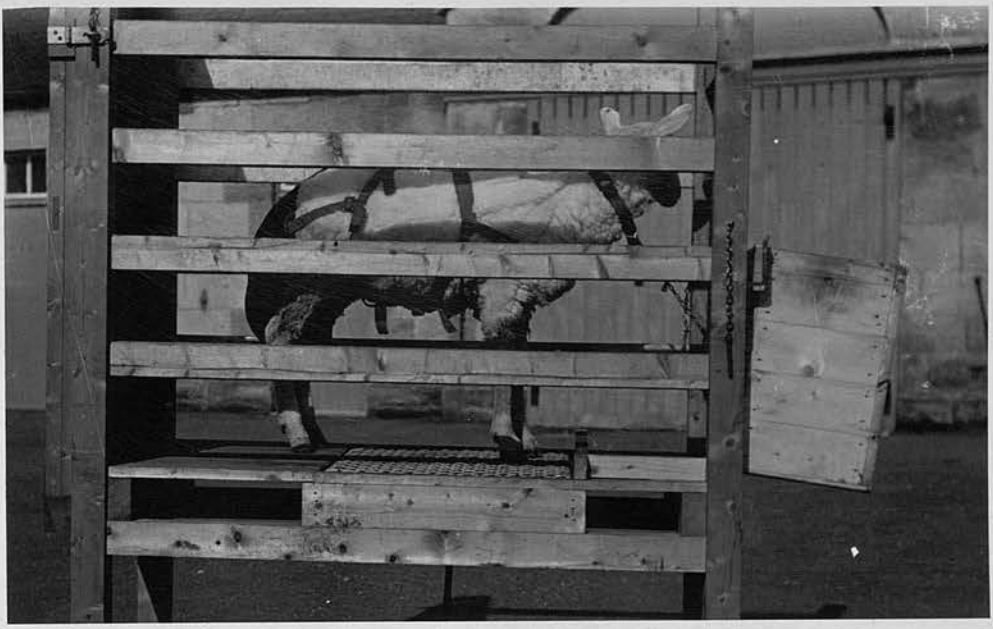


Fig. 7 - Sheep metabolic crate for collecting urine.

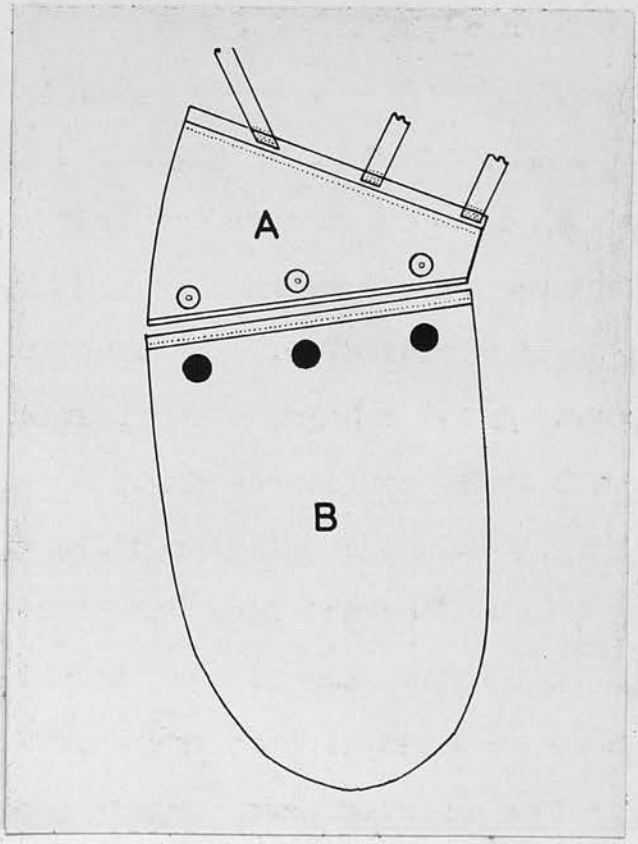


Fig. 8 - Collecting bag for sheep faeces.

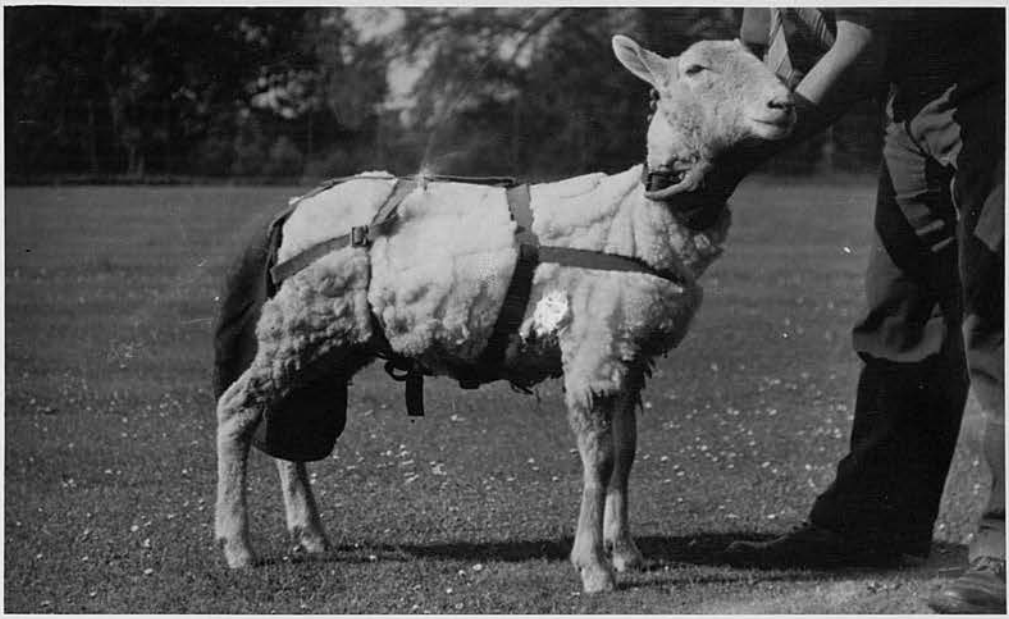


Fig. 9 - Bag for collecting faeces.

collected each morning, thoroughly mixed, measured and sampled.

Since a period of time must be allowed for the digestion and metabolism of the food consumed, the faeces and urine collected on any one day, were related to the food consumed the previous day.

Bullock 1

Throughout the experimental period small amounts of mucus were observed in the faeces. On the 14th July the bullock started grinding its teeth, scouring and shivering. Until July 15th, the temperature ranged between 101.0°F and 103.5°F but on that date

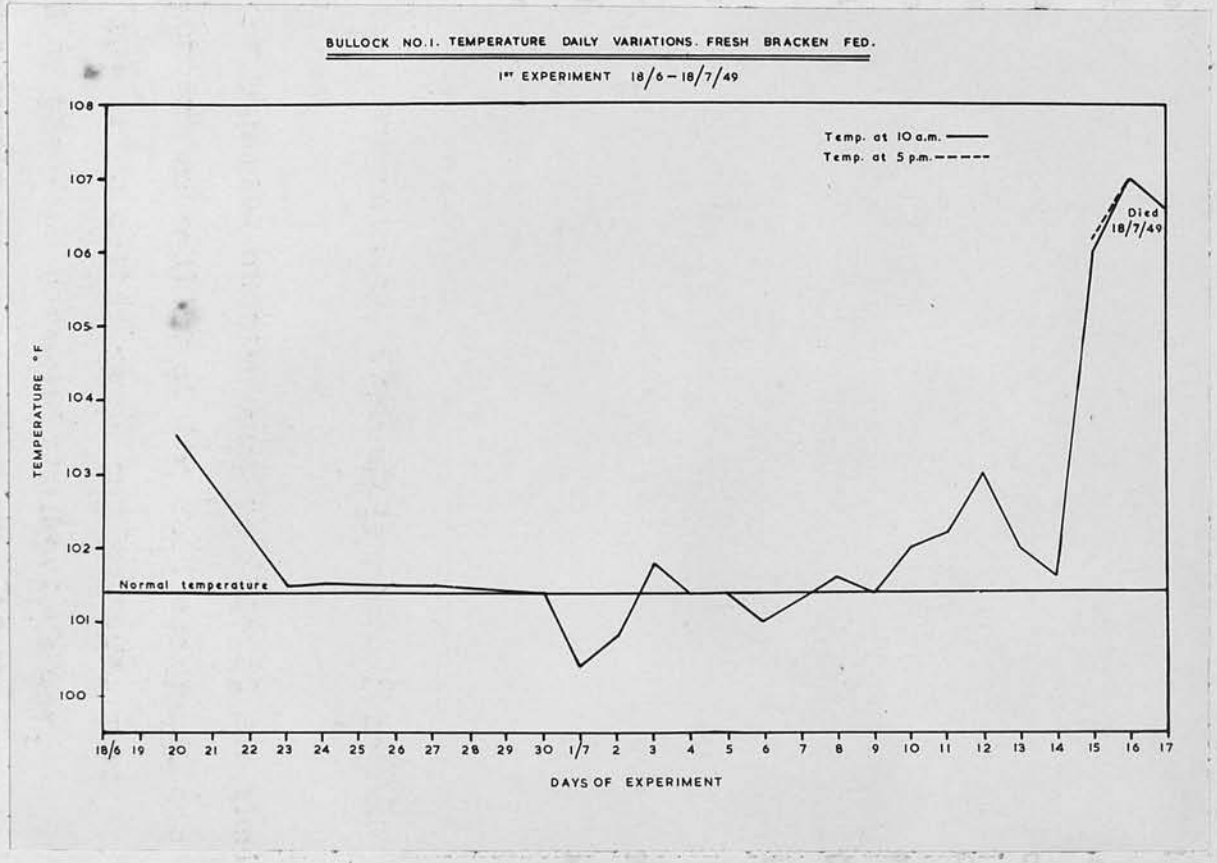


Fig. 10 - Temperature record for bullock 1.

it rose to 106.0°F and on the following day it was 107.0°F (See fig. 10).

The bullock was off its feed and shivering frequently and the nasal mucous membrane had a reddish appearance. On July 17th frothing was observed from the mouth and there could no longer be any doubt about bracken poisoning having been produced. At 1.30p.m. the bullock was injected intravenously with 22cc. 40% sodium thiosulphate and at 6.30p.m. a second injection of 7 cc. 30% sodium nitrite + 11 cc. 40% sodium thiosulphate was given. Bracken was removed and hay offered. At 9 a.m. on July 18th the bullock was found dead, with gelatinous blood clots coming from the rectum. The postmortem picture was typical of bracken poisoning. It is clear that the injection of sodium nitrite and sodium thiosulphate was of no benefit in this advanced case of bracken poisoning. Since the condition was definitely diagnosed only 48 hours before death, and the treatment applied less than 24 hours before death, recovery of the animal was hardly to be expected even if the cyanide factor is involved in producing bracken poisoning. There can be little doubt that by the time the injections were given there would already be extensive damage in some of the internal organs.

Sheep C.

The faeces of this animal showed some mucus

in the 1st two weeks but were normal afterwards. The amount of bracken eaten was very small and the live weight showed a decrease of about 1 lb per day falling from 91 lb at the start of the experiment to 58 lb at the end as shown in Fig. 11 .* No symptoms of bracken poisoning were produced. After 29 days the feeding of bracken to this animal was abandoned.

2nd Experiment

Mature, followed by young (second growth) bracken.

(Bullock 4, Sheep E and F; Sheep C and D controls)

The second bracken feeding experiment was carried out with bullock No. 4 and sheep E and F. Sheep C was fed on hay and used as a control animal for collecting normal blood samples for analysis. Sheep D was offered bracken for one week but preferred to starve; this animal became very weak and unable to stand in its cage, so was turned out to grass. On a later date some control blood samples were collected from this sheep also.

This experiment lasted 65 days, commencing on August 18th and ending on October 24th. The same procedure was employed as in the 1st experiment except that chloroform was used as urine preservative in place of toluene. Mature bracken was fed until October 8th by which date the bracken was completely brown and very dry (38% dry matter). From October

*And Appendix I page I

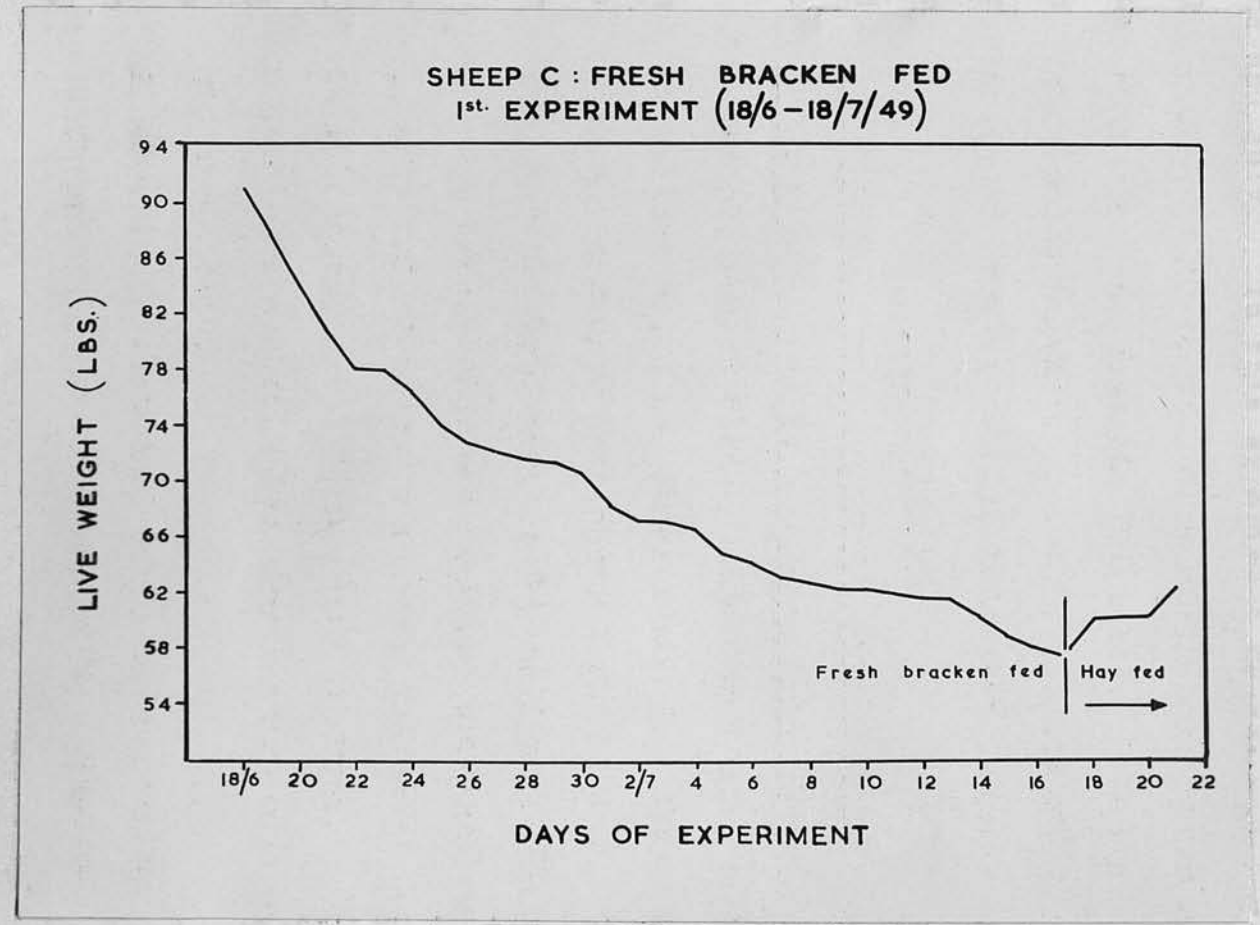


Fig. 11 - Live weight record of sheep C.

9th the animals were fed on the young second growth of bracken obtained from the area cut previously in June and July. In this experiment faeces were collected daily as well as urines. HCN was determined in the bracken fed each day and HCN and HCNS were also determined in the urine samples and periodically in the blood plasma. Weekly blood samples were collected for the determination of prothrombin time and the measurement of the specific gravities of whole blood and plasma; the latter figures were used to estimate the Haemoglobin, plasma protein, and Hematrocrit values. The urinary thiamin excretion was determined periodically and urinary and blood pyruvic acid levels were also examined.

During this experiment the bracken consumed by the three animals was as follows:-

Animal	Duration of Experiment (days)	Bracken consumed (Kg) Total	Average (per day)
Bullock 4	65	301	4.63
Sheep E	64	38	0.60
Sheep F	66	106	1.60

Bullock 4.

From the beginning of the experiment until October 19th the temperature of the bullock was usually sub-normal but ranged from 99.2°F to 102.6°F. Some small

blood clots appeared in the faeces occasionally until October 20th when many blood clots appeared and the temperature was 102.7°F. On October 21st some clotted blood was observed coming from the nose, and the bullock was shivering continuously and off its feed. At 2 p.m. the temperature was 104.4°F. Bracken was removed, hay offered and an intravenous injection of 150mg vitamin B_1 was given; the temperature rose to 105°F at 3 p.m.. On October 22nd the temperature was still high and blood clots appeared from the rectum and nose; a bleeding ulcer on one eyelid was also observed. A second injection of 100 mg vitamin B_1 was given subcutaneously at 10a.m. On October 23rd the temperature reached 105.8°F in the morning and 106.0°F in the evening as shown in figure 12. A third subcutaneous injection of 75 mg vitamin B_1 was given at 10.40 a.m. and a fourth injection of 50 mg vitamin B_1 was given at 8.45 p.m. On October 24th the temperature went down to 99.2°F and a final injection of 50 mg vitamin B_1 was given at 9.30 a.m. As shown in the following table the amounts of vitamin B_1 injected into this animal during 3 days totalled 425 mg.

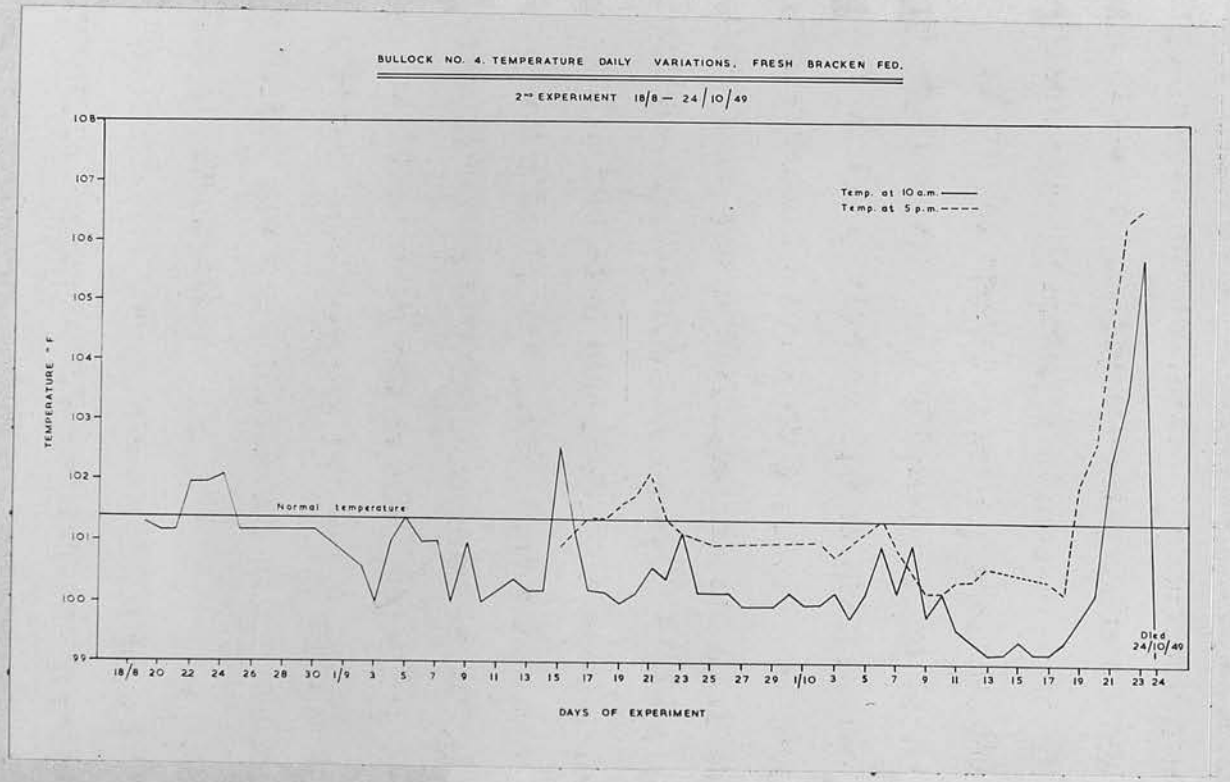


Fig. 12 - Temperature record of bullock 4.

Date of Injection	Time	Method of Injection	mg B_1 Injected
21/10	2 p.m.	intravenous	150
22/10	10a.m.	subcutaneous	100
23/10	10.40a.m.	"	75
"	8.45p.m.	"	50
24/10	9.30a.m.	"	50
Total			425

At the time of the last injection the bullock was very weak and lying down, and at 2 p.m. was found dead. Postmortem examination showed the typical symptoms of bracken poisoning; the main observations are summarised in the following table.

Fig. 13 - Bullock 4 - Postmortem appearance of heart and lungs.

1. Lungs mottled and showing blood clots.
2. Heart covered with haemorrhages and blood clots.

Organ	Postmortem appearance
Rumen	Clean inside walls, some haemorrhages on outside covering, pH of rumen contents 6.03.
Reticulum	Some haemorrhages on internal covering, pH of contents 6.67.
Omasum	Clean inside walls but haemorrhages on the outside, pH of contents 6.85.
Abomasum	Internal walls covered with haemorrhages and small ulcers. pH of contents 6.41.
Liver	Purple mottling with some internal lesions.
Lungs*	Superficial haemorrhages.
Heart*	Widespread diffuse haemorrhages on outside.
Bladder	Widespread haemorrhages on external and internal walls.
Small intestine	Internal walls had many petechial haemorrhages and some ulcers.

* See Fig. 13.

Sheep E.

This sheep showed no abnormality during the period of the experiment. Its weight was originally 75.5 lb and showed a gradual decrease for 32 days falling to 57.5lb on September 19th. From the 19th of September, until commencing to feed the second growth of bracken on October 9th, there was a gradual increase in weight up to 71.0lb (i.e. nearly 0.7 lb per day).

Following the change over to young, second growth bracken there was a rapid decline in weight until the end of the experiment when it reached 56.5 lb and death occurred. The correlation between live weight and dry matter consumed is clearly shown in figure 14 and appendix I (page 2). No abnormality occurred until the 21st October when the sheep was found lying with its head twisted to one side as shown in figure 15, it was found to be impossible for the sheep to hold its head in the normal position. No symptoms of bracken poisoning appeared and the temperature remained subnormal. It had been 101°F on October 13th and at 10 a.m. on October 21st was only 95°F. There were no signs of blood in the faeces and no other indication of haemorrhage.

Being suspected as a case of simple vitamin B_1 deficiency this animal was at 12.15 p.m. injected intravenously with 75 mg vitamin B_1 and hay was offered, but not eaten. The sheep was found dead at 2.30 p.m. Postmortem examination gave the results tabulated below; there were no signs of bracken poisoning and no subcutaneous haemorrhages.

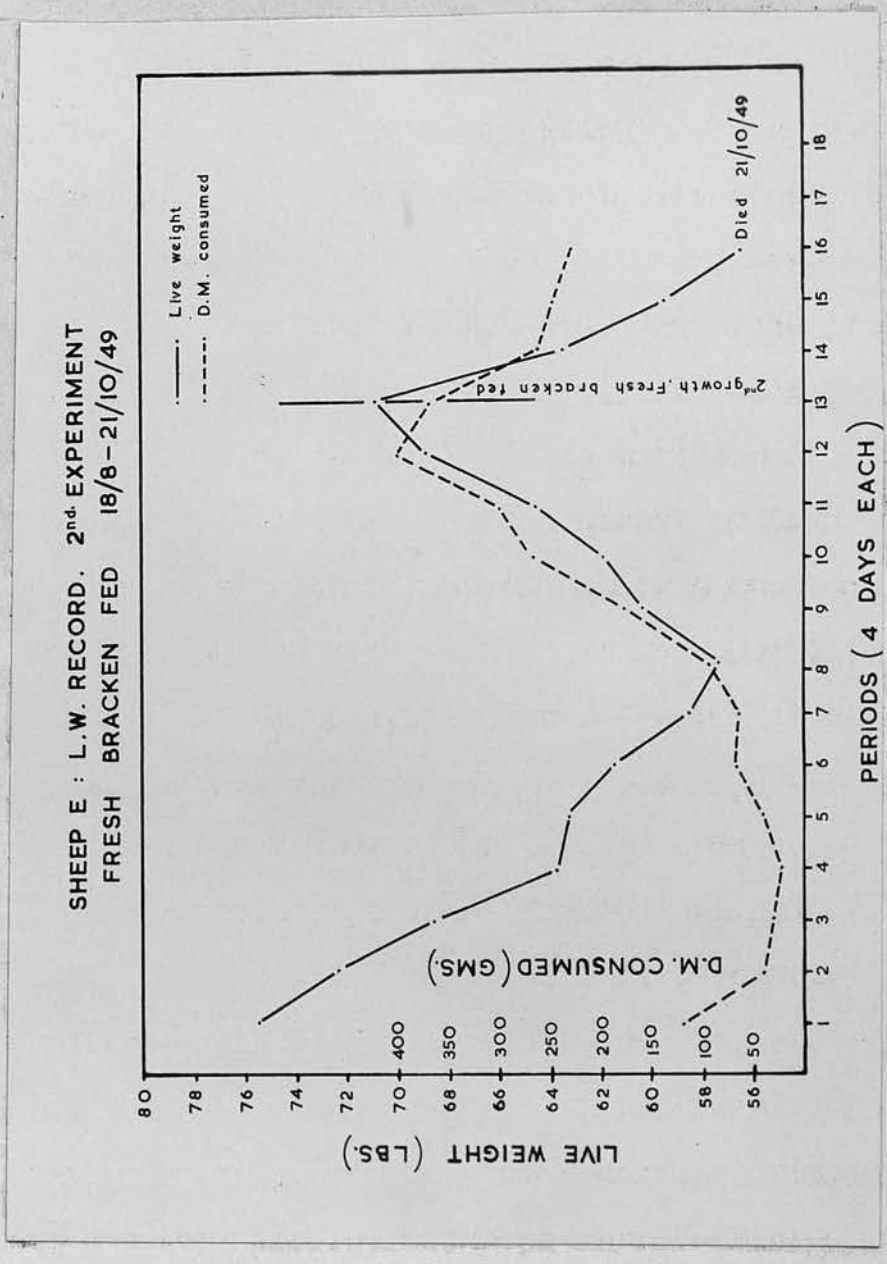


Fig. 14 - Live weight and food consumption record of sheep E.

Fig. 15 - Sheep E showing symptoms before death.

Organ	Postmortem appearance
Rumen	Walls perfectly clean with no ulceration. pH of contents 6.95.
Reticulum	Walls perfectly clean with no ulceration. pH of contents 7.32
Omasum	Walls perfectly clean with no ulceration. pH of contents 7.32
Abomasum	Walls clean except for 1 - 2 small pink spots. pH of contents 2.41
Liver, lungs, large and small intestines	Quite normal.

Sheep F.

This sheep showed no abnormality until October 12th, when the temperature increased from 102°F, recorded during the previous period, to 104.0°F. The animal was grinding its teeth and was off its feed. On October 13th the temperature was still 104°F and the faeces became very soft. The temperature fluctuated from 102.4°F to 104.0°F between October 13th and October 22nd, when considerable amounts of mucus and many blood clots were observed in the faeces, which were very soft. On October 23rd the temperature had risen to 108.0°F at 10 a.m. and was 107.0°F at 12 noon (see Fig. 16.). The faeces were very soft and of bad odour, with many blood clots. Typical bracken poisoning symptoms were observed and at 12.30 p.m. the sheep was injected intravenously with 75 mg vitamin B_1. The animal was subsequently found to be bleeding from the point of injection and the blood showed very delayed clotting so at 1 p.m. an intramuscular injection of 10 mg vitamin K analogue (menaphthone) was given. However at 1.30 p.m. the sheep was found dead.

Sheep F weighed 86.5 lb at the beginning of the experiment and dropped to 80.0 lb in 12 days. After the 16th day however there was a rapid increase. Following the feeding of the 2nd growth of bracken

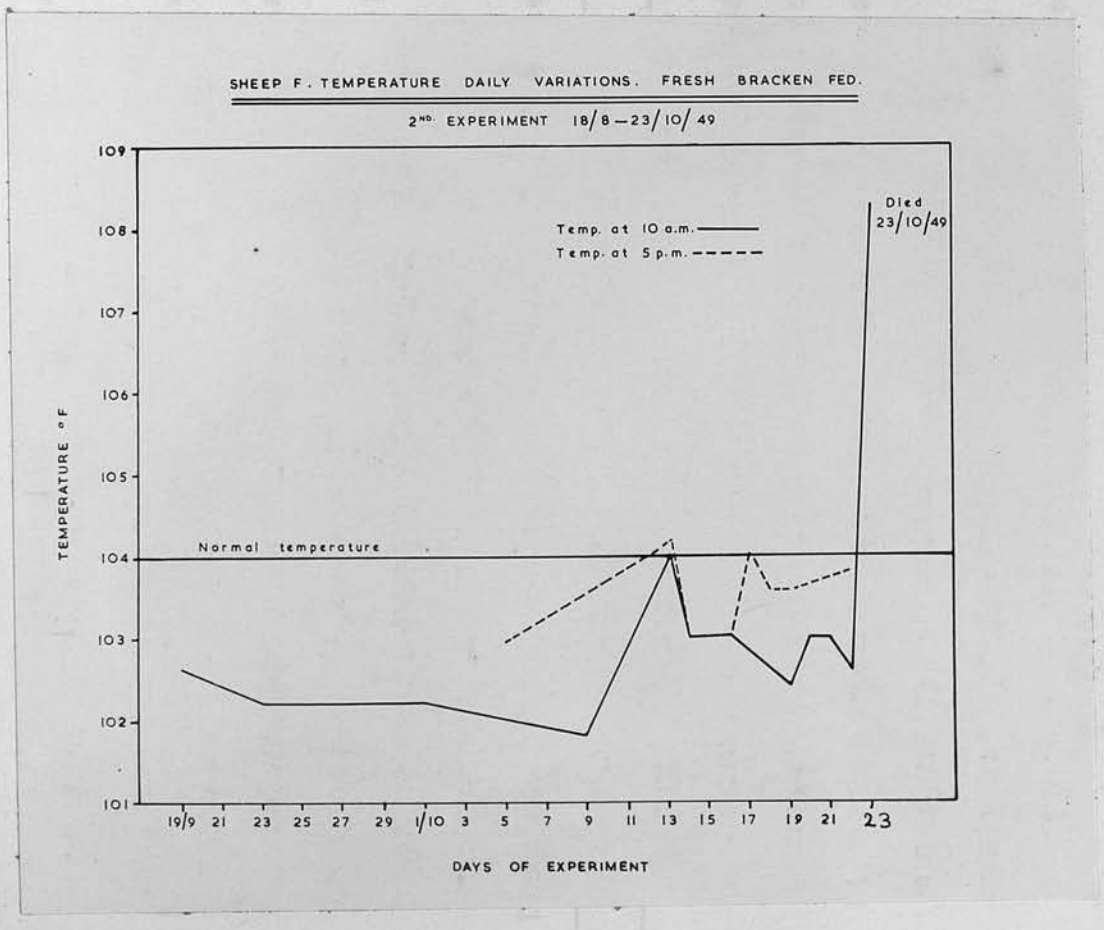


Fig. 16 - Temperature record of sheep F.

there was again a rapid decrease in weight as shown in figure 17 and appendix I (page 3). This figure shows that, as in the case of sheep E, there is a close correlation between the live weight and the dry matter consumed.

Postmortem examination showed typical bracken poisoning with many internal haemorrhages and blood clots. The principal observations are summarised in the following table.

Organ	Postmortem appearance
Rumen	Full of bracken material. Internal walls quite clean, but some pink blood spots on external walls. pH 5.87.
Reticulum	Some red spots on external walls pH 6.56.
Omasum	Internal walls clean except for a few small blood spots. pH 6.35
Abomasum	Multiple petechial haemorrhages and some ulceration of inside wall visible on outside. pH 4.48.
Liver	Mottling, but not extensive.
Kidney	Haemorrhages at centre
Spleen	Small haemorrhages.
Lungs	Purple mottled.
Heart	Superficial haemorrhages.
Small intestine	Extensive small haemorrhages on internal walls.
Large intestine	Internal walls dark coloured with some congestion of the blood vessels and extensive small haemorrhages.

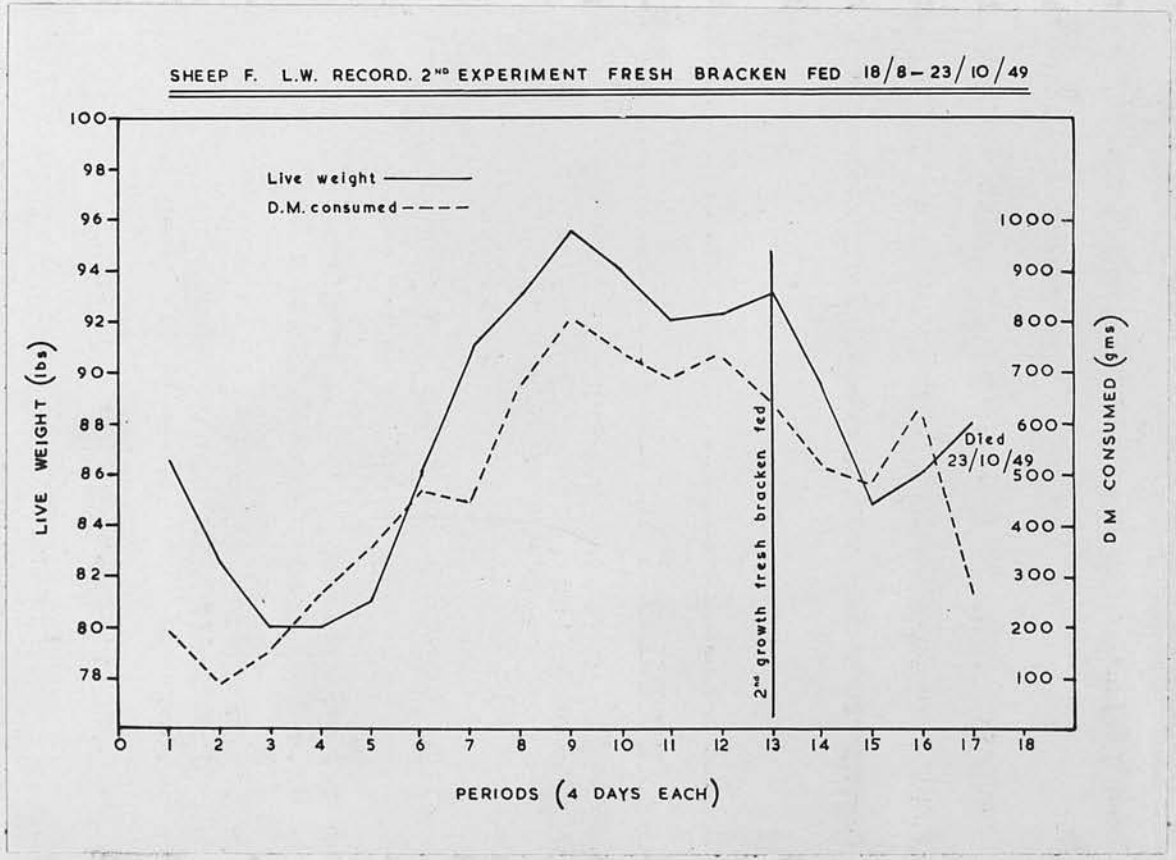


Fig. 17 - Live weight and dry matter consumption record of sheep F.

This appears to be the first clear case of bracken poisoning in sheep reported in this country and it is concluded that the only r-easons bracken poisoning does not commonly occur in sheep, as in cattle, are the more selective feeding habits of sheep and possibly their inability to consume bracken in sufficient quantity. The following table shows that sheep F, which developed bracken poisoning, consumed nearly three times as much bracken as sheep E, which did not develop bracken poisoning.

Sheep	Duration of Experiment (days)	Bracken consumed (Kg) Total	average per day	Observations
E	64	38	0.60	Died without symptoms of bracken poisoning
F	66	106	1.60	Died with bracken poisoning

Starvation may have contributed to the death of sheep E since the quantity of bracken eaten was not enough to meet the body requirements for maintenance and the animal would therefore be depleting its reserves for a prolonged period.

Comparing the bracken consumption of sheep C in the 1st experiment and sheep E in the second experiment we find that both consumed insufficient bracken to meet their energy requirements. Sheep C however, was

only kept at this very low plane of nutrition for 29 days and survived, whereas sheep E which was kept at the same starvation level for a much longer period, died after 64 days.

During the long period August 18th to October 8th when bullock 4, sheep E and F were all fed on fully mature bracken, no effects whatsoever were observed; temperatures and faeces were normal and the sheep showed increases in live weight. When, however, the animals were fed on young bracken between October 9th and October 24th bracken poisoning symptoms developed very clearly and in the very short period of about 14 days, these symptoms being followed by the deaths of all the animals.

A summary of the quantities of mature and young bracken eaten by the animals in this experiment is given in the table overleaf.

The quantities of the two types of bracken eaten per day by the bullock were very similar, yet 52 days on mature bracken had no effect although only 13 days on young bracken were needed to produce death. Sheep F also was unaffected by 52 days on mature bracken yet died after 14days on young bracken, although admittedly consuming a somewhat larger quantity per day of the younger material. There was clearly a great difference in toxicity between the two types

Bracken consumed in 2nd experiment

Animal	Type of bracken fed	Period of Experiment	No. of days	Bracken consumed Total (Kg)	Bracken consumed Average per day (Kg)	Remarks
Bullock 4	A. mature	18/8/49-8/10/49	52	242.274	4.659	Lived
	B. young	9/10/49-21/10/49	13	58.551	4.505	Died
Sheep E	A. mature	18/8/49-8/10/49	52	26.637	0.512	Lived
	B. young	9/10/49-20/10/49	12	11.155	0.930	Died*
Sheep F	A. mature	18/8/49-8/10/49	52	78.696	1.513	Lived
	B. young	9/10/49-22/10/49	14	27.631	1.974	Died

* Not with Bracken Poisoning.

of bracken. Although sheep E was fed on the same two types of bracken, death occurred without any symptoms of bracken poisoning, but the daily consumption of bracken was very low indeed and as already indicated starvation was probably at least a contributory factor in producing the death of this animal.

Dried bracken feeding experiments

Fresh bracken was cut on 30th June 1949 and dehydrated the same day in a commercial grass drier, then stored in a well ventilated hut for further use.

The feeding of chopped dried bracken

Three sheep C, D and G were used in this experiment. They consumed little bracken at first but larger quantities were eaten after a while. The total and average daily quantities of bracken consumed are shown in the following table.

Sheep	Period of Experiment	No. of days	Dried bracken consumed Total Kg	Average per day (Kg.)	Result
C	3/4-10/5/50	38	20.52	0.54	Lived
	24/6-28/6/50	5	5.07	1.01	Lived
	9/7-31/7/50	23	24.93	1.08	Lived
	Total period	66	50.53	0.76	-
D	16/3-10/5/50	56	25.80	0.46	Lived
	24/6-9/7/50	16	17.09	1.06	Died
	Total period	72	42.90	0.59	-
G	16/3-24/5/50	70	42.86	0.61	Died

Sheep C was fed chopped dried bracken in three separate periods of 38, 5 and 23 days respectively. Between the first two of these periods there was an interval of 44 days when cubed alcohol-extracted bracken was fed and between the last two periods there was an interval of 10 days when cubed whole bracken was fed. Throughout all periods no abnormalities developed in sheep C except for the presence of a little mucus in the faeces; temperatures were either normal or subnormal, ranging between 101.4°F and 102.8°F. The live weight declined at the beginning of the experiment when food consumption was low, but subsequently showed a gradual increase. When the experiment terminated on July 31st 1950, this sheep was still gaining weight and appeared quite healthy.

Sheep D This animal was fed bracken in two separate periods of 56 and 16 days respectively. Between these two periods there was an interval of 44 days during which bracken cubes were fed. During the periods when chopped dried bracken was fed, sheep D was normal except for a little mucus in the faeces; temperatures were either normal or subnormal, ranging between 102.0°F and 103.5°F during most of the time but rising to 104.4°F towards the end of the experiment. No marked change was observed until July 9th when the bracken consumption went down and the temperature suddenly

rose to 109.0°F as shown in figure 18. Blood clots and mucus were noticed in the faeces, which were very soft. At 8 p.m. the sheep was found dead, showing slight haemorrhages on the gums, some congestion on one eyelid and much blood at the anus. The postmortem findings are summarised below.

Organ	Postmortem appearance
Heart	many small haemorrhages on inner and outer walls.
Lungs	very mottled with local haemorrhages.
Liver	some haemorrhages.
Kidneys	practically normal, but dull in colour
Muscle	a few haemorrhages under the skin (not extensive)
Spleen	multiple extensive haemorrhages
Rumen	slight haemorrhages on outer walls pH of contents 6.75.
Reticulum	quite clean, content fairly fluid pH 6.9
Omasum	quite clean without haemorrhages pH of contents 8.0
Abomasum	contents appeared to be blood, pH7.4 inside walls one mass of petechial haemorrhages with possibly a few ulcers
Small intestine	extensive diffuse and petechial haemorrhages, pH of contents 7.6
Caecum	numerous haemorrhages on walls pH of contents 7.4
Colon	numerous haemorrhages some on outside walls. pH of contents 7.1

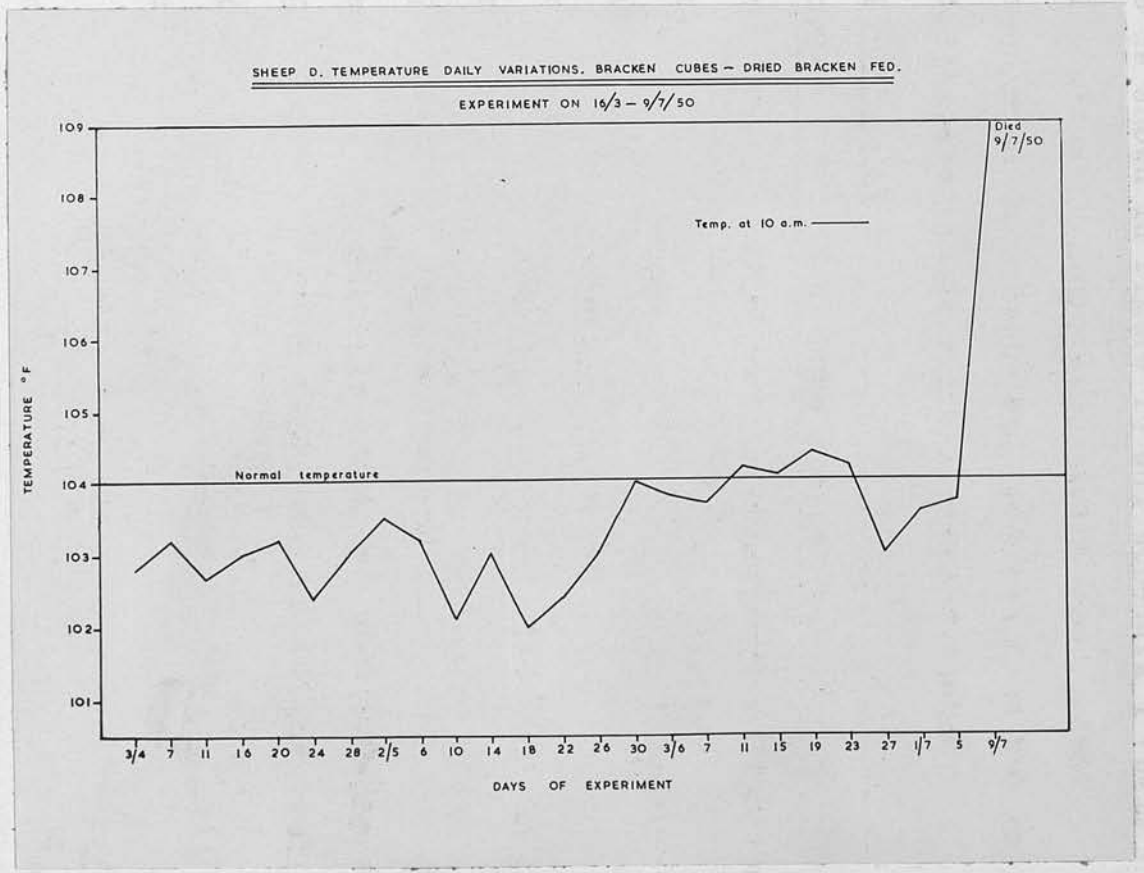


Fig. 18 - Temperature record of sheep D.

Sheep D consumed about 43 Kg dried bracken in 72 days, with an average of 0.596 Kg perday. The live weight curve is shown in figure 19 and appendix I (page 5).

There was a sudden decline in weight at the beginning of the experiment, followed by an almost continuous rise until the sheep died on July 9th.

Sheep G. was fed on chopped dried bracken for an unbroken period of 70 days and was normal from the beginning of the experiment until 16th May when some gelatinous material and blood clots were observed in the urine and mucus in the faeces. The temperature was normal at this period. No further change was observed until May 20th when the temperature rose to 106.7°F at 10 a.m. and was 106.9°F at 5 p.m. The temperature remained high until 24th May ranging between 106.6°F and 105.4°F (See Fig. 20).

On May 21st many blood clots were found in the faeces which had a very bad smell. From May 22nd to 24th the faeces were very soft and of bad odour, with blood clots present. On the latter date this sheep was found dead at 4.30 p.m. showing the typical symptoms of bracken poisoning. The post mortem findings are sumarised on page 65.

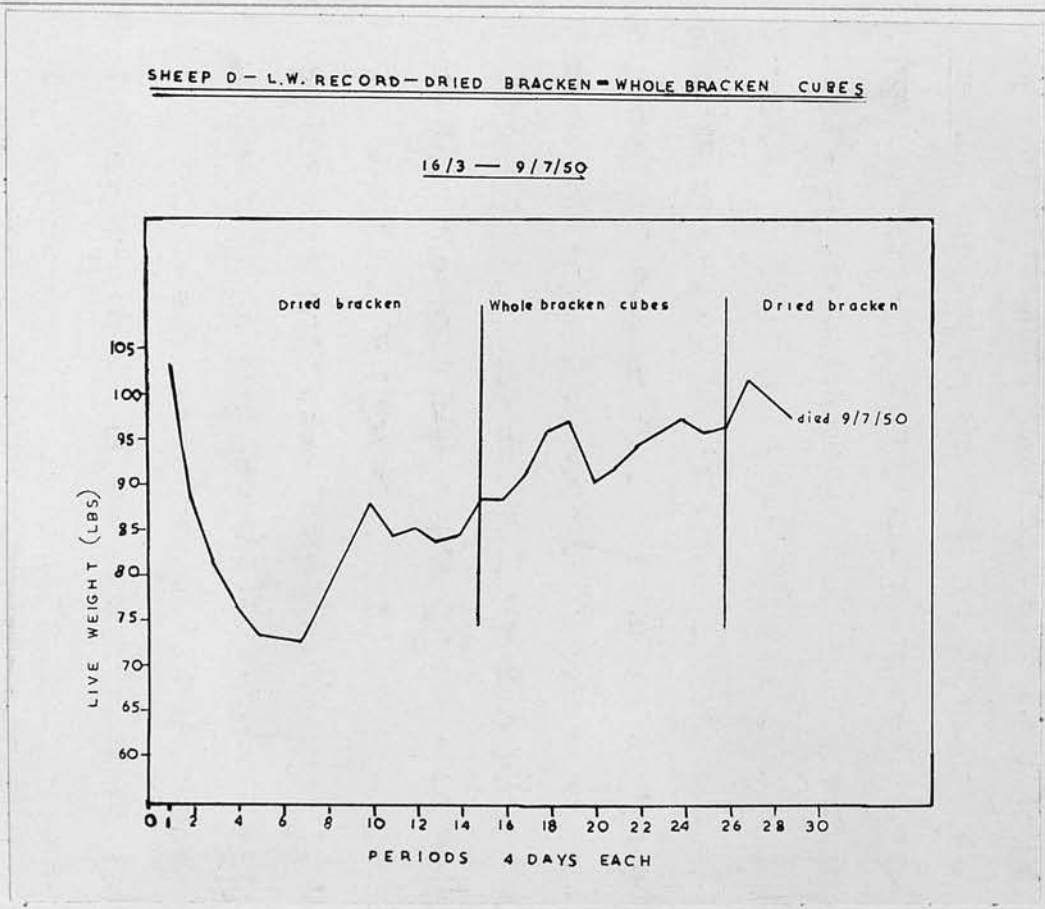


Fig. 19 - Live weight record of sheep D.

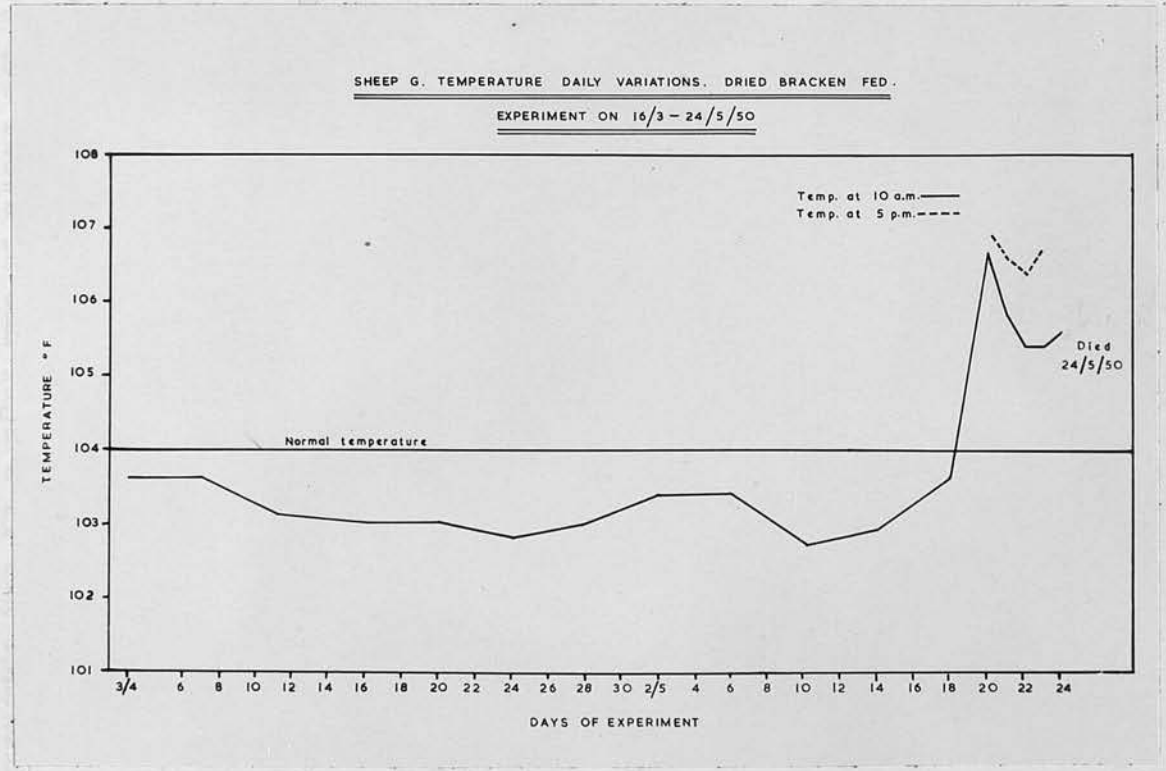


Fig. 20 - Temperature record of sheep G.

Organ	Postmortem appearance
Skin	some subcutaneous diffuse haemorrhages.
Rumen	no haemorrhages or ulceration in walls pH of contents 6.95.
Reticulum	no haemorrhages in walls pH of contents 7.00
Omasum	no haemorrhages in walls pH of contents 6.55
Abomasum	extensive mottling on inner lining of walls, some congestion and small haemorrhages and ulcers.
Liver, Kidney	normal
Lungs	extensive haemorrhages with intense purple mottling.
Spleen	a few small surface haemorrhages.
Small intestine	contents red in colour, containing blood with some clots. Extremely small haemorrhages on walls and some small ulcers.
Large intestine	some red clots of blood and petechial haemorrhages.
Heart	widespread haemorrhages.on walls.

The live weight of the sheep and the dry matter consumed are shown in ~~this~~ figure 21 and appendix I (page 4).

It will be seen that the live weight was closely proportional to the dry matter consumed, as was the case in the fresh bracken feeding experiments previously mentioned.

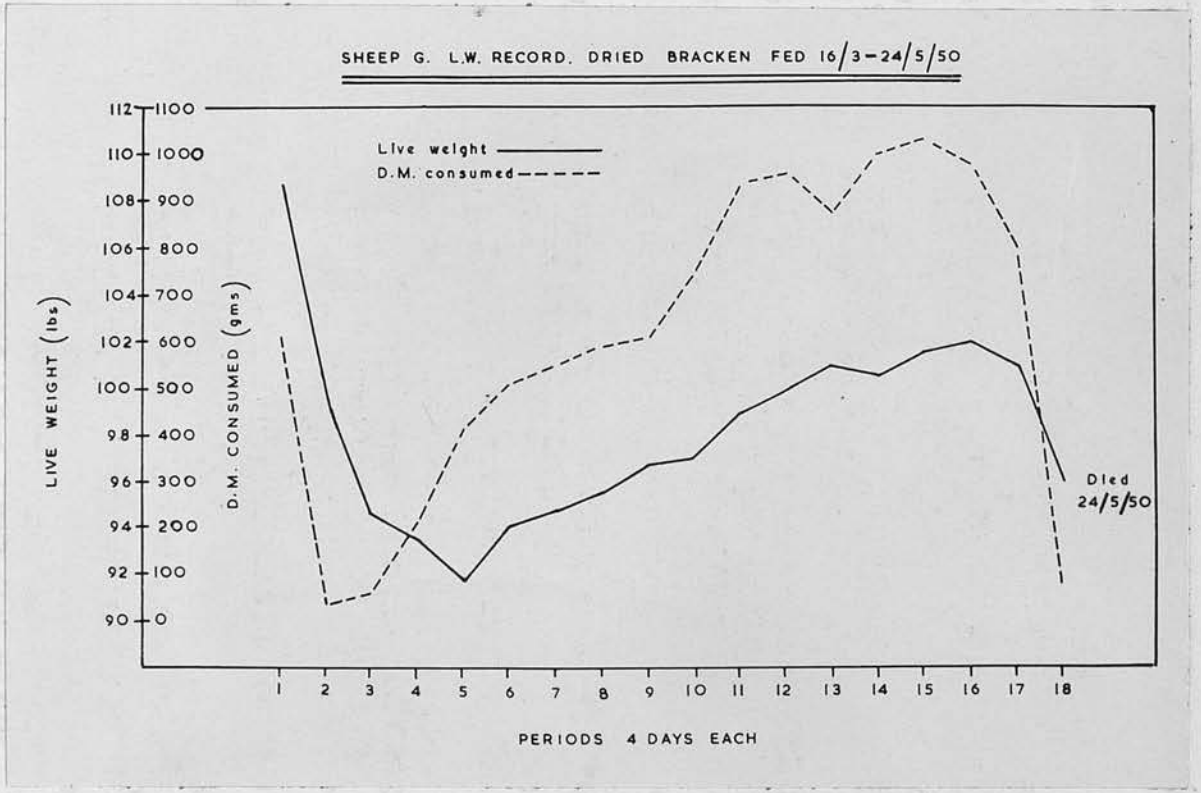


Fig. 21 - Live weight and food consumption record of sheep G.

This experiment showed that it is possible to produce bracken poisoning in sheep if sufficiently large quantites of dried bracken are consumed. Sheep G consumed about 43 Kg in 70 days with an average of 0.614 Kg per day, while sheep D consumed about 43 Kg in 72 days averaging 0.596 Kg per day.

Sheep F, in which bracken poisoning was produced by feeding fresh bracken, consumed in a similar period of time the equivalent of about 30 Kg bracken dry matter. It appears that larger quantities of bracken dry matter may need to be consumed to produce toxicity when dried bracken is fed, than when fresh bracken is used. Indeed, in the case of sheep C it will be seen that as much as 50.5 Kg of dried bracken have been fed without producing poisoning. It must be borne in mind, however, that in the feeding of chopped dried bracken to this sheep there were two intervals of 44 and 10 days respectively during which cubed bracken was fed. In the longer interval of 44 days cubes prepared from alcohol-extracted bracken were fed and if these were free from the toxic principle the interval may have provided a break in the chain of accumulation of poison, sufficient to nullify any effect from the first period of 38 days feeding on chopped dried bracken. Although in the case of sheep D there was also a 44 day interval in

which bracken cubes were fed, in this case the cubes were prepared from whole bracken and may not have had the same effect in breaking the chain of accumulation of poison.

The feeding of whole bracken cubes

Some dried bracken was hammer milled, and commercially cubed in an attempt to secure an increased consumption of bracken and a more rapid toxic effect. These cubes were fed to sheep D immediately after the first 56 days feeding on chopped dried bracken already described. In addition to the cubes a small daily feed of chopped dried bracken (250g.) was also given in this experiment to facilitate rumination.

The experiment lasted for 44 days and it will be seen from the following table that the daily consumption of bracken in cube form was considerably above the daily consumption of chopped dried bracken in the immediately preceding experiment. Nevertheless, this feeding on cubed bracken produced no toxic effects whatsoever. The live weight record in Fig. 19 (page 63) and appendix I (page) 4) shows that the 44 days feeding on whole bracken cubes led to an increase from 89 to 97 lb.

From the figures for both experiments shown on the next page it is seen that the sheep consumed about 35 Kg dried bracken and nearly double that

Consumption of bracken by Sheep D

Experiment	Period of Experiment	No.of days	Dried bracken consumed (Kg.) Total	Dried bracken consumed (Kg.) Average per day	Whole bracken cubes consumed (Kg) Total	Whole bracken cubes consumed (Kg) Average per day
Chopped dried bracken	16/3-10/5/0	56	25.804	0.461	-	-
Whole bracken cubes	11/5-23/6/50	44	9.057	0.206	60.783	1.381
Total for both experiments	16/3-23/6/50	100	34.861	0.349	60.783	0.608

quantity of whole bracken cubes in 100 days although no poisoning occurred and no abnormality appeared, apart from the presence of some mucus in the faeces.

Although 41 Kg dried bracken consumed in 70 days (average 0.583Kg per day) caused the death of sheep G and 43 Kg dried bracken in 72 days (average 0.596Kg per day) caused death also to this sheep (D), 60 Kg of whole bracken cubes consumed in 44 days (average 1.381 Kg per day) had no harmful effect on sheep D.

The lack of effect from the large consumptions of whole bracken cubes is not readily explained although it may be that the second heating involved in the cubing process has some effect on the toxicity, or the irritation of the gastro-intestinal tract produced by long bracken may be necessary for the absorption of the toxic factor.

The feeding of extracted bracken cubes

In this experiment sheep C was fed on cubes prepared from bracken meal after commercial extraction with alcohol to remove the cyanogenetic glucoside fraction. The sheep liked these cubes very much and consumed a large quantity in a very short time. After only 7 days it was eating 2 Kg per day. A small amount of chopped dried bracken (250g.)was also offered each day but was mostly refused. This experiment lasted for 44 days and followed immediately

after the 38 days feeding on chopped dried bracken described previously. From the following table it is seen that about 86 Kg of extracted bracken cubes were eaten in 44 days (average 1.953 Kg per day) which is nearly double the quantity of dried bracken which caused the death of sheep G. Nevertheless there was no ill effect of any sort and in this experiment there were not even any signs of mucus in the faeces. It seems that extracted bracken cubes have no poisonous effect on sheep even when fed at a high level. They are, furthermore, quite palatable and of definite nutritive value as indicated by the live weight gains of sheep C shown in figure 22 and appendix I (page 6).

Consumption of bracken by sheep C

Experiment	Period of Experiment	No. of days	Dried bracken consumed (Kg)		Extracted bracken cubes consumed (Kg)	
			Total	Average per day	Total	Average per day
Chopped dried bracken	3/4-10/5/50	38	20.522	0.540	-	-
Extracted bracken cubes	11/5/-23/6/50	44	4.834	0.109	85.932	1.953
Total for both experiments	3/4-23/6/50	82	25.356	0.310	85.932	1.048

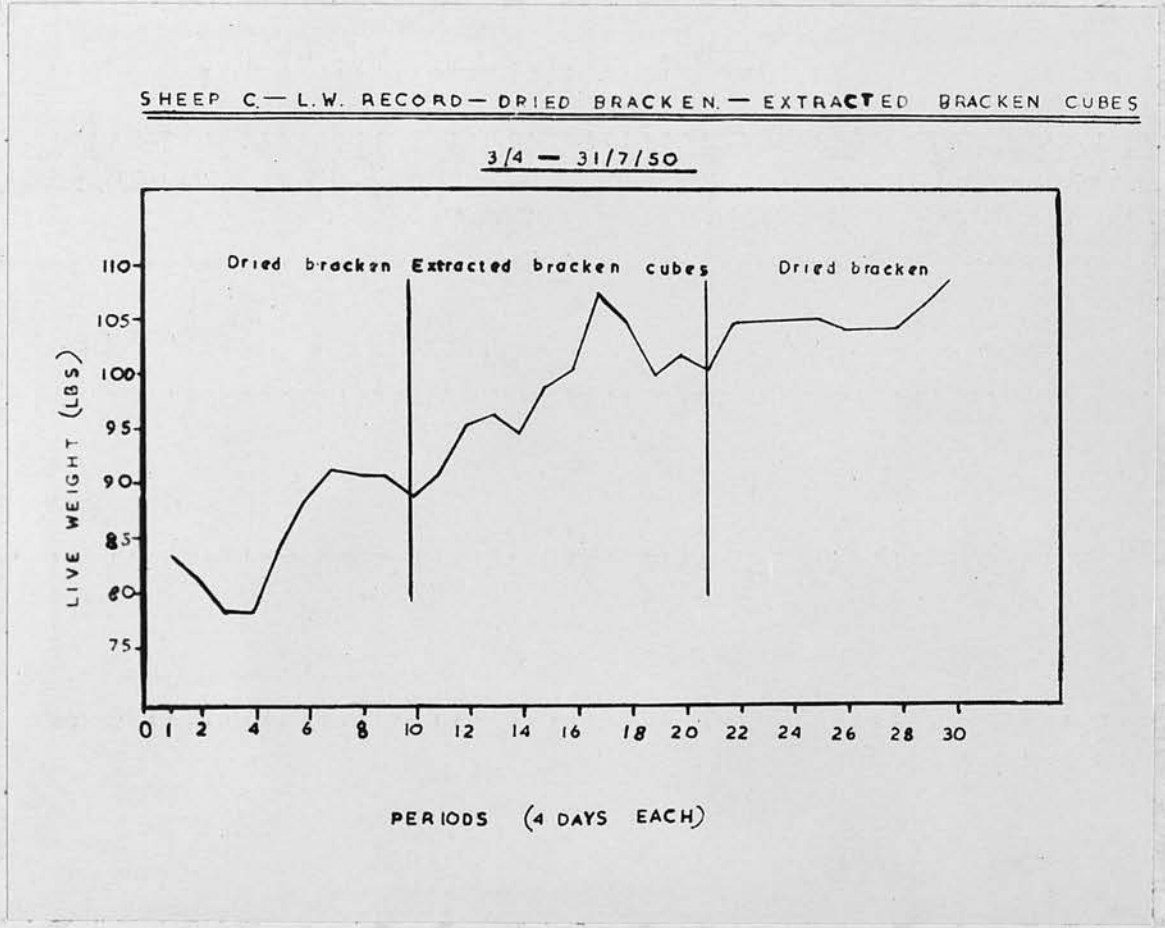


Fig. 22 - Live weight record of sheep C.

The feeding of an alcohol extract from dried bracken.

This experiment was carried out with bullock No.5 to see the effect of the alcohol extract on the animal. The extract wasavery thick dark green liquid, very unpalatble to the animal. Drying produced a toffee-like solid which was refused by the bullock even when mixed with grass meal and flaked maize. Finally the extract was warmed and poured over chopped hay, then well mixed, and the mixture of hay and extract fed. The first day the bullock ate the whole of the 10 lb of this mixture offered to it but for the next two days refused to eat any, and drank very little water. After 2 - 3 days, however it started to eat again and in 14 days the whole of the extract (obtained from 100 - 150 Kg of dried bracken) was consumed. The experiment started on 26th June and ended on July 9th without any obnormality. The temperature recorded daily was below normal, ranging between 98.8°F and 100.8°F.

Cyanide feeding experiment

This experiment was carried out with bullock No.5 and sheep H after a pre-experimental period in which both were given hay ad lib and normal samples of urine were collected for analysis. In the case of bullock No.5 an aqueous solution of sodium cyanide was absorbed in part of the feed but sheep H was given sodium

cyanide in aqueous solution as a drench. The best way of giving the cyanide in the food was found to be by absorbing the dose in grass cubes previously dried in the oven at 100°C. From 10 to 15 cc of solution were found to be easily absorbed by 100g. of grass cubes. As the bullock was fed twice daily at 10 a.m. and 5 p.m. the cyanide solution was given in two doses, each absorbed in 50 g. of grass cubes. During the preliminary experimental period of 14days the bullock was given 10 cc of water absorbed in 100 g. of dried grass cubes, in addition to hay, and the subsequent change over from water to cyanide solution did not appear to be noticed.

The quantity of cyanide solution used was sufficient to supply the bullock with a daily intake of HCN (80 to 100 mg) equal to the quantity found in the amounts of fresh bracken consumed daily in the earlier experiments (appendix 2 page 7-20). One cc of the sodium cyanide solution used was equivalent to 8.935 mg HCN, so the initial daily dose of 10 cc of this solution provided 89.35mg HCN. The amounts of cyanide given in this experiment are shown in the following table.

During the first 28 days of the experiment the bullock received the equivalent of 89.35 mg HCN per day but for the following 31 days this was increased

NaCN Administration Experiment

Animal	Period of Experiment	No. of days	Dose administered per day NaCN cc	Dose administered per day HCN mg	Total HCN intake gm.	Result
Bullock 5	15/3-11/4/50	28	10	89.35	2.5018	Lived
	12/4-12/5/50	31	15	134.03	4.1548	Lived
Sheep H	15/5-31/7/50	78	6	54.108	4.2204	Lived

to 134 mg per day. The bullock consumed its normal diet of hay and showed no ill effect, although it received in 59 days a total amount of sodium cyanide equivalent to 6.6566 g. HCN.

The experiment with sheep H showed the same negative result although the dosing with cyanide lasted for 78 days. This animal was drenched twice daily, at 10 a.m. and 5 p.m. with 3 cc aqueous sodium cyanide solution each time, providing the equivalent of 54.108 mg HCN per day. This quantity of HCN is similar to that found in the amounts of bracken consumed daily by sheep F, which subsequently died from bracken poisoning. The animal consumed its normal diet of hay and showed no ill effect, although it received in 78 days a total amount of sodium cyanide equivalent to 4.22 g HCN.

With both the animals used in this experiment no abnormality of any sort developed. There was also no change in the urinary thiocyanate excretion as a result of the HCN dosing and the significance of this finding will be discussed in a later section.

In order to detect the respiratory excretion of HCN, sheets of paper treated with sodium picrate were fixed to a wire netting frame and suspended close to the nose of the bullock for 24 hours; after this time some brick coloured areas were observed,

indicating the release of some HCN in respiration. However, the amount of HCN released in this way could not be estimated.

Summary Part II

The following points may be presented as a summary of the results of the feeding experiments carried out.

1. The ash of mature bracken has no poisonous effect even when consumed in relatively large amounts.
2. The poisoning of animals fed on bracken appears to be closely related to the stage of growth, young bracken being most toxic.
3. It has been shown for the first time that bracken poisoning can be produced experimentally in sheep as well as in cattle and horses, although it is often difficult to induce sheep to eat sufficient bracken. The apparent absence of natural cases in sheep is probably due to their more selective grazing habits.
4. Bracken poisoning in sheep produces the same symptoms and post-mortem picture as in cattle.
5. Bracken poisoning may also be produced in sheep by feeding artificially dried bracken although a long period of consumption was found necessary in these experiments.
6. The live weights of animals fed entirely on bracken were proportional to the quantities of dry matter consumed. When sufficient bracken (fresh or dried) was consumed its nutritive value was clearly demonstrated by the gains made.
7. Whole bracken cubes did not show any poisonous

Part II

Was feeding of ark worthwhile? Prev. analysis of bracken ark revealed no evidence of toxic constituents.

pp 40 & 41. Expt 1 Block C

Was feeding bracken continued long enough? was it certain (established) that carcinogen had a weight was fairly nutritious?

Expt 2. What does liver wt — feed consumption correlation mean - don't follow this?

Pre Block E what fact dec of starvation ??? P.T.O

diagnosed without doubt.

p 54

What are these daily mean consumption figures worth

p 62 Consumption of Sheep
- how does this compare with Sheep E reputed to have died of starvation.

p 70 Hens stumped over the bracken cubes.
- explanation not convincing

p 71 - very interesting

effect when fed to a sheep at a relatively high level (1.38 Kg per day) for 44 days.

8. Extracted bracken cubes fed to a sheep for a similar time and in even larger amounts (1.95Kg per day) had no poisonous effect and were quite palatable.

9. The feeding of an alcohol extract of dried bracken for a relatively short period of time did not produce bracken poisoning, although it was obviously distasteful to the animal.

10. The feeding of pure cyanide salts in quantities providing amounts of HCN equivalent to the amounts found in toxic bracken, failed to produce poisoning. This does not entirely rule out the possibility that the glucosidal HCN of bracken plays some part in the production of bracken poisoning.

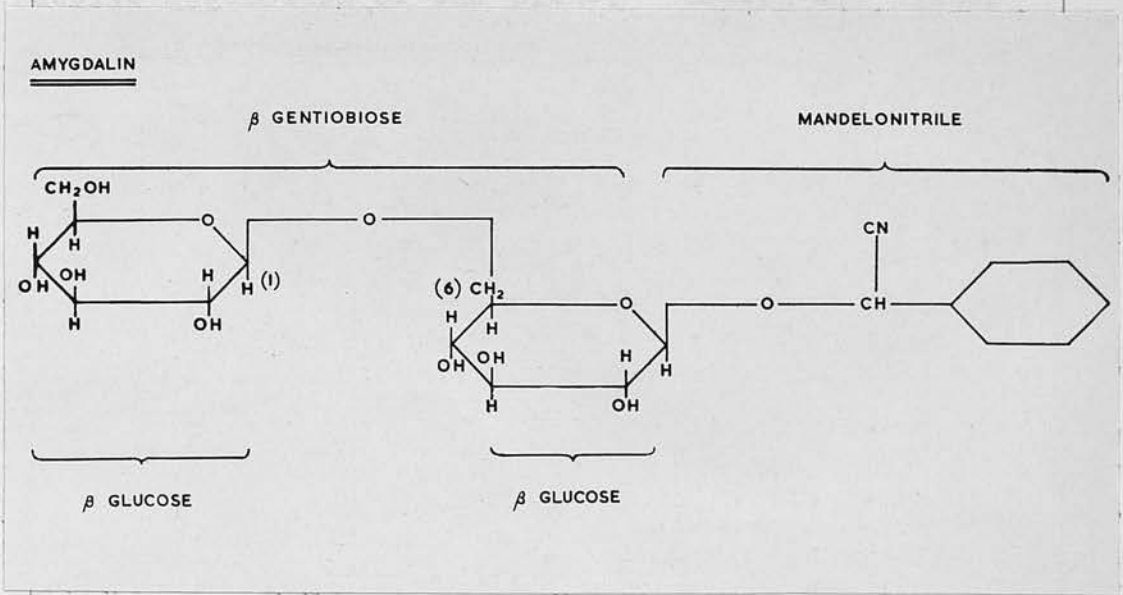
11. The injection of relative large amounts of vitamin B_1 failed to cure animals showing bracken poisoning and injections of sodium nitrite and sodium thiosulphate were also ineffective. It seems doubtful whether any treatment could be successful at the late stage when bracken poisoning can be diagnosed without doubt.

Part III

The significance of the cyanide fraction in bracken and its relation to bracken poisoning

A The occurrence and significance of cyanide compounds in plants

In the vegetable kingdom hydrocyanic acid most probably represents a stage in the metabolism of nitrates, which are absorbed from the soil and eventually built up into plant proteins. The hydrocyanic acid occurs either free or more usually in combination in the form of cyanogenetic glucosides like amygdalin, found in bitter almonds.



The almond seed also contains the enzyme emulsin, and on macerating the seed with water, emulsin is brought into contact with the dissolved glucoside and causes decomposition to glucose, benzaldehyde and

hydrocyanic acid according to the following equation:-

$$C_6H_5CH(CN)\text{-}O\text{-}C_6H_{10}O_4\text{-}O\text{-}C_6H_{11}O_5 + 2H_2O$$
$$= C_6H_5CHO + HCN + 2C_6H_{12}O_6.$$

Treub (64) has concluded that the accumulation of cyanide in the hair cells and oxalate cells is an indication that its formation is normally related to the photosynthetic process. The relationship between the activity of chlorophyll and the production of glucosides has been postulated by many workers who believe that glucosides are of importance in the active metabolism of the plant. Godwin and Bishop (31) working on Cherry Laurel, found that cyanogenetic glucosides form a large fraction of the carbohydrates stored in the leaves. The glucoside is formed as a normal anabolic product and is not intended to be a protective compound or a definite food reserve. The glucoside, when split by an enzyme, liberates sugar for use in respiration. According to Ravenna and Zamorani (49) nitrogen passes through the following stages in the plant:-

Nitrate ⟶ HCN ⟶ amino-compounds ⟶ protein.

According to this theory the cells which are actively involved in protein synthesis use HCN for the building of protein substances. Leeman (37) has also concluded that HCN is an intermediate product in plant metabolism and this hypothesis is accepted by many other workers

in the field. All investigators have found that the amounts of glucosides in plant are affected by many different factors, the most important of which are as follows:-

1. Type of soil

Heavy soils and soils rich in nitrogen or treated with nitrogenous fertiliser have been found to increase the hydrocyanic acid content of sorghum and millet plants. (9,38,52)

2. Species of plant

Some species of Acacia are relatively rich in prussic acid, whilst others are not (59). The hydrolysing enzyme may also be differently distributed in different species and toxicity only occurs when plants providing both glucoside and enzyme are consumed. Cyanogenetic glucosides occur in many different plant species and in recent years glucosides have been isolated from Lotus Australis by Finnemore et al (20) and from white clover by Melville and Doak (40).

3. Part of the plant

The quantity of glucoside present in plants varies widely, and it is unevenly distributed throughout the whole plant, occurring chiefly in the leaves (71)

4. Stage of maturity

All investigations have shown that the amounts

of HCN in the plant decrease as the plant matures. (41,60).

5. Wilting and stunting

Wilting of the plants is accompanied by an increase in HCN content. Menaul and Dowell (41) stated that HCN does not exist as free HCN in Sudan grass but it begins to be liberated as soon as the plant is macerated or undergoes wilting. Liberation of HCN is intimately associated with enzyme action. Avery (5) reported that the amount of HCN is greater in stunted plants but Alway and Trumbull (3) found that yellow stunted plants contained less of the acid than the green, vigorous plants in the same field.

6. Infection

Willaman and West (72) carried out experiments on Sorghums, and found that unhealthy plants usually contained more HCN than healthy ones. The unhealthy condition may be due to malnutrition, to improper transpiration, or to insect attack. It is possible that under such conditions the plant produces more glucosides for the purpose of stimulating hormone action. Balfour (6) also noticed that Sorghum attacked by aphis sorghi contained more HCN than unaffected plants.

7. Climate and season

Willaman and West (72) stated that the apparent

effect of humidity and temperature on the amount of HCN in Sorghum is probably due to an indirect effect on the rate of growth. An adequate water supply is usually accompanied by low HCN content. Menaul and Dowell (41) found that more HCN was found in the summer than in the winter whilst Franzke (23) reported that sorghum strains sampled on a warm bright clear day showed different HCN variations than when sampled on a cool cloudy day.

8. Freezing

The belief that HCN is developed in Sudan grass by freezing (60) is attributed to the bursting of the green cells liberating the enzyme and acting in the same way as maceration, with the result that the HCN is rapidly lost from frosted grass.

9. Diurnal variations

Many investigators have examined the diurnal variations in the HCN content of Sorghum and other plants (23.41,45,49,50, 56). In nearly all cases it has been found that there is an increase during the early part of the day when photosynthetic activity is intense, and a rapid decline at night. Different strains of the same species of plant may vary in the extent of their diurnal variations.

For studying the toxicity of a plant containing a cyanogenetic glucoside it is essential to consider

the concentrations of both the glucoside and the hydrolysing enzyme, since both are needed for the liberation of HCN. Some plants may contain the glucoside but no enzyme, whilst others may contain the enzyme without the glucoside; yet others may contain both glucoside and enzyme, whilst some may contain neither of these substances (12). Seddon and King (54) reported that the sheep on certain natural pastures may gather sufficient enzyme to split a minimum fatal dose of a cyanogenetic plant, itself containing no enzyme.

B. The toxicity of cyanides to the animal body.

1. Cases of cyanide poisoning

Cyanides may be administered to animals in many different ways, e.g. by inhalation, by intravenous or subcutaneous injection, by admixture with the feed, or by administration in capsule form or as a solution given as a drench or by stomach tube. Maximum and minimum lethal doses of cyanides differ according to the species of animal and to the method of administration. The following table summarises the doses of cyanide given to animals by different investigators.

HCN administered to sheep and cattle by different investigators

Animal	Investigator	Dose given	Effect produced
Sheep	Steyn (58)	2.2mg HCN per Kg body weight) 1 mg HCN per lb body weight)	Death
	Van Der Walt (66)	1.1mg HCN per Kg. body weight 1.6mg HCN per Kg. body weight	None Death
	Couch et al (14)	1.05mg HCN per Kg. body weight 2.29mg HCN per Kg. body weight	None Death
Cattle	Dun (16)	1.461g. HCN 1.949g. HCN	None Death
	Steyn (58)	0.5g. NaCN given as drench	Poisoning symptoms (Recovered after injection of $NaNO_2$
	Tocher (62)	0.777g. HCN	None

HCN administered to pigs, dogs, rabbots, and horses by different investigators

Animal	Investigator	Dose given	Effect produced
Pigs	Tocher (61)	0.386g. HCN in capsules	Death
	Tocher (62)	0.162g. HCN 0.3392g. HCN	None Death
	Couch et. al (13)	2.4 - 3.4 mg. HCN per Kg. body weight	Minimal lethal dose
Dogs and Rabbits.	Kaufmann (33)	0.039g. HCN	Toxic
	Steyn (57)	0.01 - 0.016mg KCN per Kg. body weight per day for 25 days	None
	Worden (74)	A daily dose of 144mg. HCN	None
Horses	Kaufmann (33)	0.389g. HCN	Toxic
	Dun (16)	0.389g. HCN	Death
	Schubel (53)	0.3888g. HCN	Death

Most of these experiments were carried out for studying the metabolism of cyanides in the animal body, the effects of cyanides in poisoning animals, or the symptoms of cyanide poisoning in comparison with the symptoms following the consumption of cyanogenetic plants.

Steyn (58) fed cut clover to a bullock after 12 hours fasting and the feed was supplemented with a drench containing 0.5 g. of NaCN in a pint of water. Within a minute symptoms of cyanide poisoning were seen, there being rapid respiration, quivering and weakness. The animal rested on the stall with its head down. As symptoms advanced a 7 ml injection of a 30% solution of sodium nitrite was given intravenously and brought about an immediate recovery.

Tocher (61) was able to cause death to pigs in 30 minutes following the administration in capsules of KCN equivalent to 0.386g. of HCN but not when the KCN was mixed with the food. This was due to the fact that the animal refused the food for several hours by which time volatilisation with the production of formic acid was probably complete.

$$HCN + 2H_2O = HCOONH_4$$

$$2HCOONH_4 + H_2SO_4 = 2HCOOH + (NH_4)_2SO_4.$$

Tocher in 1939 (62) conducted an experiment on pigs using linseed cake and HCN capsules. Two pounds of

linseed cake were fed to a pig for two days, yielding approximately 0.181g. of HCN; the pig showed no ill effect. When two capsules containing 0.162g. HCN were administered to a pig there was also no evidence of poisoning but after administering capsules containing 0.3992 g. of pure HCN, the pig died in half an hour. Tocher also conducted an experiment on 4 bullocks fed on linseed cake to ascertain whether sufficient HCN would develop to cause illness or death. The four bullocks were each fed for 7 days on 15 lb turnips and 2.5 lb straw, in addition to 0.75 lb of linseed cake developing 0.02% HCN. Thus each bullock consumed 0.068 g. HCN. For another seven days each bullock was fed on 1.5 lb linseed cake developing 0.311 g. HCN. Then for 14 days he fed each animal 2.5 lb linseed cake developing 0.521 g. of HCN; no ill effects were observed. In the last experiment Tocher administered potassium cyanide in capsules to the four bullocks and found that an amount equivalent to 0.7776 g. of HCN had no ill effect.

Gettler and Baine (27) found no trace of HCN in fresh normal human tissues (brain, liver, kidneys, lungs) and blood even th-ough the test used was sensitive to 0.01 mg HCN in 100 g. of tissue, but when alkali cyanides were taken orally, and death followed within 10 - 15 minutes, over 70% of the

cyanide taken was found in the gastro-intestinal tract. Where HCN was inhaled, traces might be found in the stomach and intestinal walls but the higher content occurred in the lungs. The average absorbed lethal dose of cyanide found in humans at the time of death was 1.4 mg. HCN per Kg body weight.

Worden (74) found that foodstuffs fumigated by HCN were highly toxic to the rabbit and dog. He then administered sublethal doses to rabbits and dogs without ill effect. HCN consumed in eight daily doses of 0.2 gm. of fructose cyanhydrin, each equivalent to 18 mg. HCN, had no ill effect.

Hurst (32) injected monkeys intramuscularly with daily increasing quantities of 0.2% aqueous solution of KCN. On postmortem examination the brains of many monkeys showed no definite microscopic changes whilst in other cases lesions were observed.

Van DerWalt (66) used a stomach tube for dosing sheep twice daily with an aqueous solution of KCN. The potassium cyanide solution first used was equivalent to 1.1 mg. of HCN per KG. body weight, which is near the minimal toxic dose; later this was increased to 1.6 mg HCN per Kg. body weight, which is near the maximum toxic but not lethal dose. Of the five ewes receiving 1.6 mg. HCN per Kg body weight four died suddenly, with acute HCN poisoning.

2. Symptoms of HCN poisoning

Symptoms appearing in animals poisoned by hydrocyanic acid may be due to acute or chronic poisoning.

a. Acute poisoning

Symptoms in acute HCN poisoning reported by Gettler and Baine (27) are sudden collapse, with or without convulsions, followed by death in 2 - 5 minutes; with relatively small doses (5 - 100 mg KCN) even 10 minutes may elapse before symptoms of staggering, palpitation, dyspnea, feeling of oppression in the chest and dilated pupils, are observed. In sheep Van Der Walt (66) stated that the symptoms observed are often severe and consist of apathy, severe dyspnoea, accelerated pulse and twitching of the lips.

b. Chronic poisoning

Koelsch (34) reported in factory workers exposed to HCN, the symptoms of dizziness, dryness of the throat, sense of pressure in the gastric region, increased haemoglobin value, normal colour index of the blood, and a slightly increased percentage of lymphocytes and basophile leucocytes.

Weike (70) observed vomition, oppressive feeling in the chest, tremors of the arms and legs, and sleeplessness. Sometimes the blood showed no significant changes.

Couch and Bunyea (13) found that the symptoms appearing in pigs poisoned by HCN consist of an increase in the rate of respiration, often accompanied by a spasmodic blinking of the eyes; the respiration rapidly becomes shallow and rapid, The animal moves aimlessly as if anxious, staggers and falls on its side, then rises again. This rising and falling may be repeated several times before the animal finally collapses, sinks into a coma and dies in respiratory paralysis.

Schubel (53) observed that the head is turned towards the side when the animal lies down, the pupils of the eyes are dilated and a watery discharge is given off. The tongue is partially paralysed and large amounts of saliva run from the mouth. The limbs and ears are cold. In the last stages, the limbs are paralysed and death occurs after unconsciousness.

3. Detoxication of cyanides and treatment of HCN poisoning.

a. Metabolism and detoxication of cyanides

Cyanides are metabolised in the animal body with the production of thiocyanates. Giacosa (28) and Adeline (1) stated that when the CN group is attached directly to an aromatic ring it is probably

at least partly hydrolysed in vivo to COOH and ammonia, but where the CN group is separated from the ring by a CH_2 group, it is split off as HCN, which is then detoxicated to thiocyanate as shown below. When the CN group is attached to an alkyl group HCN is also produced.

$ArCN \longrightarrow ArCOOH + (NH_3)$ (aromatic group)

$ArCH_2CN \longrightarrow ArCOOH + HCN \longrightarrow HCNS$ (aromatic group)

$RCH_2CN \longrightarrow RCOOH + HCN \longrightarrow HCNS$ (alkyl group)

Nichols (43) has mentioned the excretion of thiocyanates by the animal organism and Shohl (55) found that human saliva contains on the average 0.01% of thiocyanate. According to Trasoff and Schneeberg (63) normal blood contains about 1.31 mg KCNS per 100 c.c.

The formation of thiocyanic acid in vivo is an enzymic process involving the enzyme rhodanase (36). This enzyme occurs in nearly all animal material except blood and muscular tissue. In the dog it reaches its highest concentration in the suprarenal glands followed by the liver, which in view of its size contains the major part of the body's rhodanase supply. Dog liver rhodanase has an optimum activity at about body temperature (38°C) and at a pH of 8.3.

Lang (36) found that dried frog liver contains more

than fifty times as much of the enzyme per 100 g. as does dog's liver, whereas the rabbit has twenty times, the cow ten times and the human being three times as much as the dog. The results of Mukerji and Smith (42) showed that injected cyanides were converted into thiocyanates much less efficiently in the dog than in the rabbit. The thiocyanate produced is completely excreted within 24 to 48 hours.

The enzyme rhodanase is specific for hydrocyanic acid which is converted very rapidly in the presence of sodium sulphate or colloidal sulphur into thiocyanic acid. Certain sulphur containing organic compounds such as cystine, cysteine, glutathione, and thioethanolamine, can act as substrates for this enzyme but the amount of thiocyanic acid produced is only 0.1 to 1.0% of that formed with sodium thiosulphate or colloidal sulphur.

According to Lang (36) the reaction between HCN and $Na_2S_2O_3$, catalysed in vitro by rhodanase, does not require oxygen.

$$HCN + Na_2S_2O_3 = HCNS + Na_2SO_3$$

In the body the sulphite is rapidly oxidised to sulphate. The compound or compounds which provide sulphur for the detoxication of cyanide in vivo are unknown. Lang (36) points out that thiosulphate has never been detected as a metabolic product in

man, although the recent work of Vassel et al. (67) showed that dogs excrete thiosulphate as long as they are fed, but if food is withheld thiosulphate excretion ceases. These workers used thoroughbred bull terriers and found a marked difference in the thiosulphate output of males and females. As blood does not contain rhodanase, thiosulphate is unlikely to have any action on HCN in the blood stream, but on the other hand, when the HCN of the blood reaches the tissues it is converted to non-toxic thiocyanate by thiosulphate in the presence of the rhodanase of the tissues. It is also possible that cyanmethaemoglobin formed in the blood liberates HCN in other tissues and this is then detoxicated by thiosulphate, catalysed by rhodanase. Etteldorg et al (19) have shown that cyanmethaemoglobin on injection into dogs is converted to functional haemoglobin, methaemoglobin probably being formed as an intermediate product.

b. Treatment of HCN poisoning

There are many more efficient antidotes for oral poisoning than for the inhalation of HCN. Sub-cutaneous or intravenous injection of the remedy should be made in order to achieve rapid action.

Wolfgangwirth (73) showed that oxidising agents such as H_2O_2 or $KMnO_4$ are helpful as well as ferrous

sulphate, calcium nitrate, and sodium thiosulphate solutions. Steyn (58) used 7 ml of a 30% solution of sodium nitrite to give immediate recovery to a bullock.

In 1935 Couch et al (14) found that a thiosulphate mixture composed of 1 g. sodium nitrite and 2 g. sodium thiosulphate in 15 c.c. water was 50% effective as an antidote.

Schubel (53) in 1939 suggested the following mixtures for intravenous injection in cases of HCN poisoning.

1. 30% sodium thiosulphate +
   10% " nitrite.
2. 1% methylene blue +
   25% Dextrose +
   10% soidum thiosulphate.
3. 30% sodium thiosulphate +
   2% " nitrite.

Chen et al (10) found that intravenous injection of sodium nitrite, followed by sodium thiosulphate, was most effective in treating poisoning caused by hydrocyanic acid. They also found that when these two substances are used together there is a definite potentiation of action.

Van Der Walt (66) treated sheep which showed ataxia and collapse with 4.0g. sodium thiosulphate

given intravenously in 20% aqueous solution. Recovery after sodium-thiosulphate injection was extremely rapid, even in extreme cases.

C. The metabolism of the cyanide constituents of bracken

1. Methods of analysis

Greshoff (30) in 1908 mentioned the presence in young bracken shoots, of a glucoside, which by hydrolysis or enzyme action produces hydrocyanic acid. A study was therefore made of the content of hydrocyanic acid in bracken and its metabolism in the animal body. Various methods of HCN determination are available but many of them are either of low sensitivity or only suitable for detection rather than quantitative determination.

Qualitiative tests for HCN include the picric acid, prussian blue and copper benzidine reactions. The Picric acid test (46) involves the use of papers soaked in yellow sodium picrate, then dried; these turn orange then brick red in the presence of HCN. The modified prussion blue test of Rathenasinkam (48) dispenses with distillation, a strip of filter paper with drops of ferrous sulphate and sodium hydroxide being suspended in the material for 10 minutes. It is then immersed in dilute HCl when a blue stain appears in the presence of HCN. In the copper benzidine reaction a filter paper treated with benzidine in acetic acid and then with dilute copper acetate solution produces a bluish-violet colour with HCN. It is

necessary to use a control test paper kept in the air of the laboratory, and the difference in the time required for the production of the colour in the two papers is noted.

The quantitative determination of HCN in cyanogenetic plants and biological materials involves three different steps:-

1. sampling and preservation of the material,
2. quantitative release and separation of the HCN,
3. measurement of the released HCN.

1. Sampling and preservation of plant material

The problem of sampling and preserving cyanogenetic plant tissues in a form suitable for further investigation is of fundamental importance. During drying, living plants continue to respire and other changes also take place; these will continue until all enzyme action has been stopped. Under these conditions compounds liberating HCN may suffer partial or total destruction. Furthermore fresh cyanogenetic plants stored at room temperature without preservation lose 13 - 83% of their HCN content in 1 to 6 days (8). In the case of animal tissues decomposition rapidly destroys HCN. Gettler and Baine (27) did not agree with the use of formaldehyde as a preservative of samples for HCN analysis whilst Briece and Couch (8) and Davies et al (15) found that when alcohol is used as

the preservative the recovery of HCN from the plant is incomplete. Briece and Couch (8) introduced the use of 1 - 2% mercuric chloride solution as a preservative for cyanogenetic plants. This reagent was also used for animal organs by Van Der Walt (65, 66). Davies et al (15) used the freeze drying method of preservation placing the fresh sample in a Kilner Jar surrounded by solid powdered carbon dioxide at a temperature of -80°C in a thermos flask. The sample froze rapidly and was then dehydrated under vacuum.

In the present work six different methods of preserving bracken for HCN determinations were tried. A quantity of fresh bracken was chopped and thoroughly mixed then samples weighed for the following different treatments which were carried out in duplicate as far as possible.

1. 100g. were homogenized with 200cc water, then transferred to a 500 cc Kjeldahl flask, and 1g. of tartaric acid dissolved in 2.5cc water, added. The flask was tightly stoppered and the contents allowed to macerate for 24 hours before releasing the HCN by the method of Van Der Walt (66) and measuring it by the alkaline titration method (46).

2. 200g. were placed in a glass bottle and frozen in a thermos containing solid carbon dioxide then left

for 24 hours. As the weight of the sample did not show any change in 24 hours it was then divided into two parts which were separately homogenized with 200 cc water and treated as in procedure 1 above.

3. 100g. were placed in a wire basket and autoclaved at a pressure of 20 lb per sq. inch (260°F) for 10 minutes then dried in an oven at 100°C for 24 hours. After grinding to pass a 1/16" sieve, 5 to 10g. quantities of the dried sample were macerated with 100 cc water in a Kjeldahl flask and treated as in procedure 1.

4. 400g. were oven dried at 100°C for 24 hours then milled and 10 g. macerated with 100 cc water and treated as before.

5. 100g. were macerated with 200cc of 1.5% mercuric chloride for 4 days, then homogenized and treated as before but with the addition of 2g. stannous chloride before aerating to release HCN.

6. 400g. were air-dried at room temperature then milled and treated as before.

Determinations were made on bracken collected from Carnethy hill on May 16th and from Boghall farm on May 19th 1949 and were repeated on June 7th with the exception of the mercuric chloride and autoclave treatments. The results are shown in the following table.

HCN content of bracken and changes resulting from preservation treatments

No	Treatment		% gain or loss of HCN Boghall	% gain or loss of HCN Carnethy hill	Boghall hill bracken HCN mg/100gm 19/5/49 A	Boghall hill bracken D.M. 7/6/49 B	Carnethy hill bracken HCN mg/100gm. 16/5/49 A	Carnethy hill bracken D.M. 7/6/49 B
1	Immediate homo-:genisation and maceration		-	-	41.79	38.08	28.66	34.14
2	freezing	A	Nil	-7.92	41.79	40.90	26.39	35.67
		B	+7.41	+4.48				
3	Autoclaving and oven drying		-66.69	-74.56	13.92	-	7.29	-
4	Oven drying	A	-42.25	-46.37	24.12	21.81	15.37	19.42
		B	-42.72	-43.11				
5	Macerating in 1.5% mercuric chloride		-85.61	-85.80	6.01	-	4.07	-
6	Air drying	A	-45.89	-47.11	22.61	14.10	15.16	12.50
		B	-62.98	-63.38				

It will be seen that the figures obtained for the freshly homogenised and macerated bracken and for the frozen bracken were very similar but the other treatments produced considerable losses of HCN. These results indicated that is is preferable to determine HCN in the fresh bracken, overcoming the difficulty of sampling the bulky and stemmy material by first chopping then mixing and withdrawing a sub-sample for homogenization with water. Homogenization also helps to release HCN from the plant tissues. Determinations of HCN in bracken were therefore made daily on the freshly chopped material just before feeding the animals.

II. Quantitative liberation of HCN from plant material

The release of HCN from plants can be achieved (1) by boiling with acids, (2) by autolysis (maceration with water) or (3) by adding a suitable enzyme. Williaman et al (72) in 1916 found that when macerated tissues were distilled with H_2SO_4 the distillate was yellow in colour and this interfered with the colorimetric determination of the HCN. Boiling organic materials with acid is an unsatisfactory mechanical procedure and Roe (51) stated that with amygdalin this method leads to low recoveries due to further hydrolysis of the cyanogen product,

with loss of N as NH_3. The procedure now preferred for plant materials is maceration with water for several hours, allowing the natural enzyme hydrolysis to liberate the HCN (46, 72). Askew (4) found that maceration of the ground sample in water at room temperature for 24 hours, or with incubation at 45°C for 5 hours, gave the highest yield. It may be necesaary to add a suitable enzyme prepartion during the maceration process; Roe (51) used this procedure for the determination of HCN in amygdalin, adding emulsin to promote the release of HCN.

After hydrolysis the liberated HCN may be quantitatively separated from the material by, distillation in vacuo, direct distillation, steam distillation, or aeration. Melville et al (39) found that distillation in vacuo requires a consider-:able time to remove the HCN quantitatively and so gives low recoveries. They also got low and inconsistent results by the aeration and vacuum distillation methods and came to the conclusion that for the complete removal of HCN from clover a temperature of 100°C is necessary. Steam distillation for the recovery of HCN from plant materials has been used by Greene (29), Finnemore et al (21), Briece and Couch (8) and the A.O.A.C (46). Elsdon et al (17) also used this method to obtain HCN from blood. Franzke

et al (24) in a study of cyanogenesis in Sorghum found that the aeration method gave lower values than the distillation method but Gettler and Baine (27) and Van Der Walt (66) chose the aeration method since it reduces the volume of distillate and reduces the quantity of foreign volatile organic substances in the distillate. Moreover Van Der Walt (66) found that there is no danger of formation of HCN, and no significant loss of HCN in the aeration method. He used the aeration method for both plant and animal tissues and raised the following objections to distillation either direct or with steam.

1. The volume of the distillate will be very great.
2. Distillation may cause a serious loss of HCN.
3. Great care is needed to avoid the formation of traces of HCN.
4. Volatile interfering substances may appear in the distillate.

The following method of Van Der Walt (66) was used to release and liberate the HCN from bracken in the present work.

50g. of freshly chopped bracken are weighed and homogenised with 100 cc water then transferred quantitatively to a 500 cc Kjeldahl flask using a further 100 cc water for washing. 2.5 cc 20% tartaric acid are added and the flask immediately

stoppered with a rubber stopper, the contents being left to macerate for 24 hours at room temperature.

The contents are then aerated, using the apparatus employed by Van Der Walt (66) as shown in figure 23. The macerated material in the Kjeldahl flask C is immersed in a boiling water bath B and connected to a condenser F through a splash bulb D. The condenser outlet G is connected to the absorption tubes H each containing 10 cc of 0.5% NaOH and surrounded by iced water in bath L. Aeration is produced by suction from a water pump connected to the absorption tubes at K the air being kept free from CO_2 by passing it through a 30% solution of NaOH in flask A. Air is drawn through the apparatus at a rate sufficient to agitate the contents of the distilling flask, but water is not allowed to distil over into the absorption tubes H, as this would increase the final volume of the test solution and so lower the sensitivity of the method. After aerating for $1\frac{1}{2}$ hours, the condenser tube G is washed with the minimum amount of water and the two absorption tubes H removed. The contents are transferred to a 50 cc measuring flask, and the solution then made up to the mark, thoroughly mixed and used for colorimetric determination of the HCN. In order to determine whether the addition of tartaric acid before

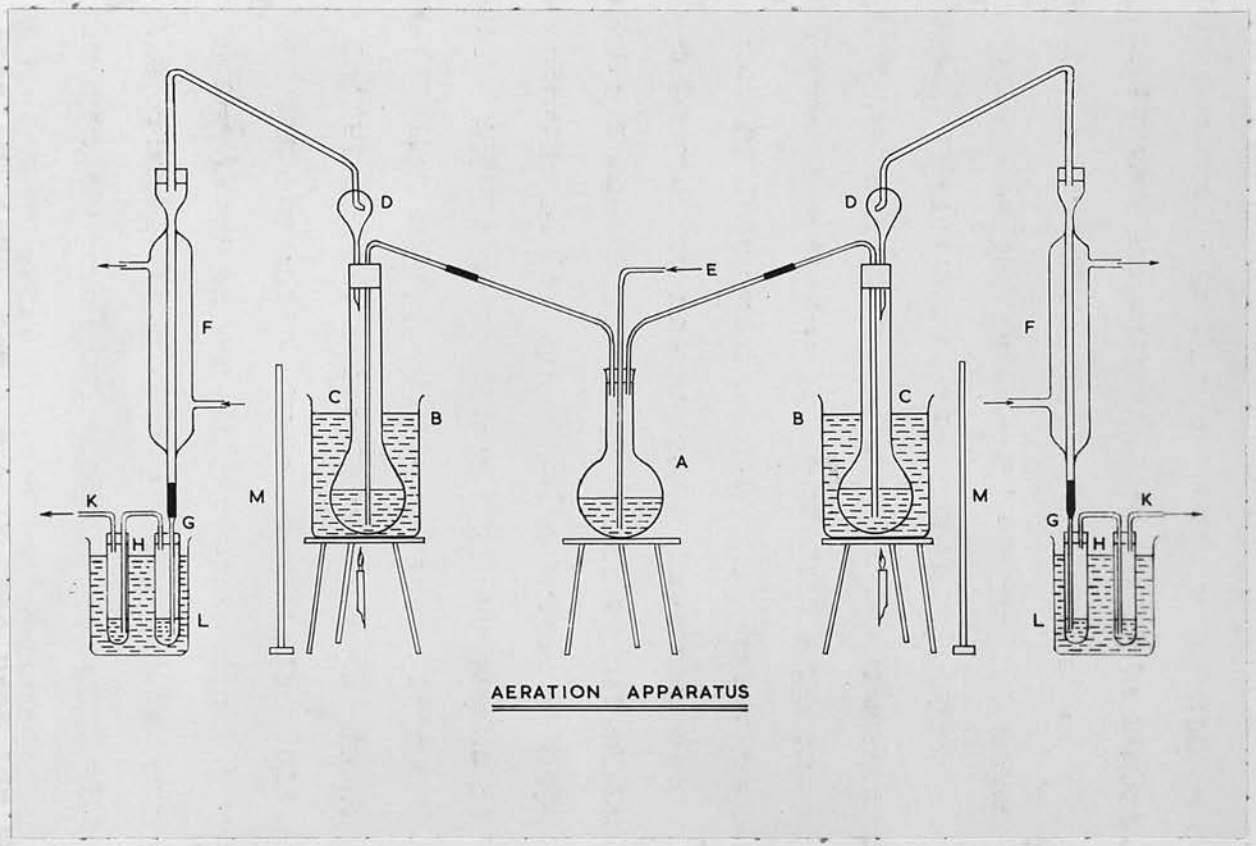


Fig. 23 - Aeration apparatus for liberation and collection of HCN.

maceration was superior or inferior to addition just prior to distillation four bracken samples were analysed by both procedures with the following results.

Bracken sample	No.	mg HCN / 100 g D. M. tartaric acid added before maceration	after maceration
Boghall hill 19/5/49	1	41.54	40.54
	2	42.04	41.30
Carnethy hill 16/5/49	3	27.80	28.15
	4	29.51	30.65

As there was no difference in the results obtained by the two procedures it was decided to add the tartaric acid before maceration in subsequent analyses.

III Measurement of released HCN

The most important methods suitable for the quantitative determination of HCN are the following:-

a. Reaction with $AgNO_3$

Since cyanides react with $AgNO_3$ producing insoluble AgCN, standard $AgNO_3$ has commonly been used for the titrimetric determination of HCN. The distillate carrying the HCN may be collected in $AgNO_3$ acidified with HNO_3 and after filtration the excess of $AgNO_3$ may be determined by titration with KCNS. Alternatively

the distillate may be collected in alkaline solution (2.5% NaOH) and this titrated with $AgNO_3$ in the presence of NH_4OH and KI to a turbid end point. Although 0.02N $AgNO_3$ may be used in these methods the sensitivity is limited by the difficulty of detecting the end point with very dilute solutions. Amperometric titration used by Laitinen et al (35) may give increased sensitivity. It is claimed that the end point of an amperometric titration of cyanide with silver nitrate can be obtained with an accuracy of 0.1 to 0.2 per cent for cyanide concentrations as low as 0.002N, which is comparable in accuracy with the visual end point.

b.- Picric acid method

This colorimetric method, used by Waller (69) in 1910, depends on the reaction between cyanide and picric acid, the colour developed being matched with a series of standards.

c. Prussion blue test

This test is specific for cyanide in the absence of ferrocyanides and was first suggested by Francis and Connell (22) and Viehoever and Johns (68) for estimating the HCN in Kafir corn. It depends on the formation of a ferrocyanide when an alkaline solution of an easily decomposable cyanide is warmed with a solution of ferrous sulphate. On subsequent

acidification with HCl and addition of a small quantity of ferric salt the deep colour of Prussian blue is produced and is matched with a series of standards. Though this test is specific for cyanides, it is not particularly sensitive and according to Swanson (60) there is difficulty in obtaining a uniform blue colour.

A modification has been developed for the determination of micro quantities of HCN in insect and plant tissues by Fulton and Van Dyke (26).

d. Thiocyanate method

Menaul and Dowell (41) estimated the HCN in Sudan grass by conversion to thiocyanate and the production of a permanent red colour with ferric chloride solution, this colour being compared with a series of standards.

e. Phthalin method

This method which according to Childs and Ball (11) is a very sensitive one, depends on the production of a red colour when a cyanide and a weak solution of a cupric salt are added to an alkaline solution of Phenolphthalin. The colour is due to oxidation of phenolphthalin but the reaction is not specific for cyanides since ferricyanides and halogens, are also

able to oxidise phenolphthalin under the same conditions, and so give a positive reaction. Nicholson (44) modified this method for determining HCN in plants and found the Phthalin prepared by the reduction of O-Cresolphthalein to be superior to Phenolphthalin.

f. Pyrazolone method

This method described by Epstein (18) is based on the conversion of cyanide into cyanogen chloride by chloramine T solution. The cyanogen chloride then reacts with pyridine to form glutaconic aldehyde which produces a blue coloured dye with 1 phenyl, 3 methyl, 5 - Pyrazolone. The colour may be measured spectrophotometrically.

g. Benzidine method

This is a colorimetric method described by Aldridge (2) in 1944 for the determination of micro quantities of cyanide and is based on the conversion of cyanide into cyanogen bromide.

$$HCN + Br_2 = CNBr + HBr$$

$$KCNS + 4Br_2 + 4H_2O = KBr + CNBr + H_2SO_4 + 6HBr$$

After removal of excess of bromine with sodium arsenite/the cyanogen bromide is allowed to react with a solution of benzidine in dilute pyridine, to give an intense orange to red colour, the intensity of which is proportional to the amount of cyanogen

bromide present.

After a preliminary examination of the methods suitable for the measurement of HCN the Benzidine method of Aldridge (2) appeared to be the most sensitive one and was therefore employed in the examination of bracken and other biological materials where the concentration of HCN was frequently very low.

IV Determination of HCN and HCNS in bracken and animal material

Bracken

For HCN determination 50 g. of freshly chopped bracken are weighed, homogenized, macerated, and aerated as described on pages 109 - 111. The benzidine method described below is then used to measure the HCN released.

Reagents

1. Saturated bromine water
2. 1.5% sodium arsenite
3. Pyridine reagent - prepared from 25cc of pure redistilled pyridine + 2cc of conc. HCl, diluted to 100 cc with water.
4. 2% benzidine hydrochloride, in water acidified with HCl. Benzidine acetate may also be used.
5. Glacial acetic acid.

Procedure

1— To 5 cc of the solution containing up to 15 µg of

HCN and made acid with a few drops of acetic acid, are added 2.5 cc of saturated bromine water and 2.5cc of 1.5% sodium arsenite. solution.

2 - at this stage the solution is left stoppered for 1 - 2 hours. 2 cc of the solution are then added to a mixture of 10 cc of pyridine reagent and 0.4cc of 2% benzidine hydrochloride solution.

3 - the orange colour immediately produced soon changes to red. After standing 10 minutes at room temperature for colour development, the solution is examined in a Spekker absorptiometer using a Hilger No. 6 filter.

In the method as described by Aldridge (2) an Ilford micro 2.303 blue filter is recommended but the absorption characteristics of these two filters are similar as shown by the percentage transmission figures shown on the next page.

For the preparation of calibration curves a solution of NaCN was standardised by the alkaline titration method of the A.O.A.C. (46) and suitable dilutions prepared. The results obtained are shown in Fig 24.

Wavelength in ang-strom units	percentage transmission blue filter micro 2.303	Hilger blue filter No.6	Wavelength in ang-strom units	percentage transmission blue filter micro 2.303	Hilger blue filter No.6
4000	-	36.7	5400	16	36.7
4200	-	46.1	5600	2.0	23.1
4400	30	54.8	5800	-	13.0
4600	56	59.5	6000	-	5.8
4800	65	61.0	6200	-	2.3
5000	59	60.0	6400	-	0.8
5200	41	54.2	6600	-	0.2

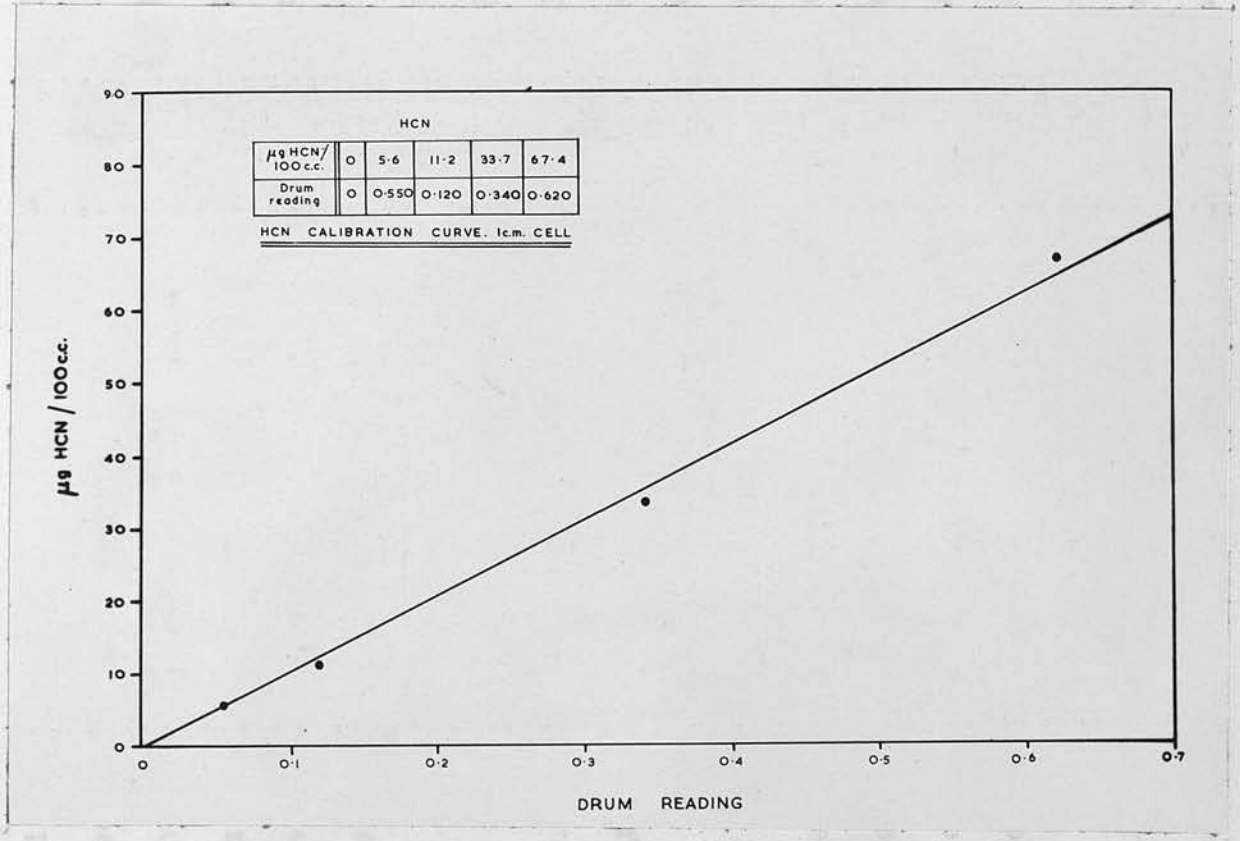


Fig. 24 - HCN calibration curve.

Urine 200 cc of urine are transferred to a 500 cc Kjeldahl flask and acidified with 15 - 20 cc conc HCl. The flask is then immersed in the boiling water bath of the aeration apparatus Fig 23 and aerated for 1½ hours. The HCN solution in the absorption tubes is transferred to a 50 cc measuring flask and completed to the mark. Aliquots are then taken for HCN determination by the method already described for bracken. The urine left in the flask after the aeration process is used for the determination of HCNS since this is non-volatile. The urine after aeration is cooled, and filtered. The flask and filter are washed with water and the combined filtrate and washings made to 250 cc in graduated flask. After mixing, aliquots are taken for the determination of HCNS by exactly the same procedure as used for measuring HCN.

A calibration curve Fig. 25 for the thiocyanate determination was prepared from suitable dilutions of KCNS standardised by the alkaline titration method (46).

Blood Plasma is obtained by drawing blood into a tube containing neutral potassium oxalate as anti-coagulant, centrifuging and pipetting off the supernatant plasma.

Serum is obtained by drawing blood into a tube without anticoagulant, allowing it to clot and pipetting off the serum after centrifuging (47).

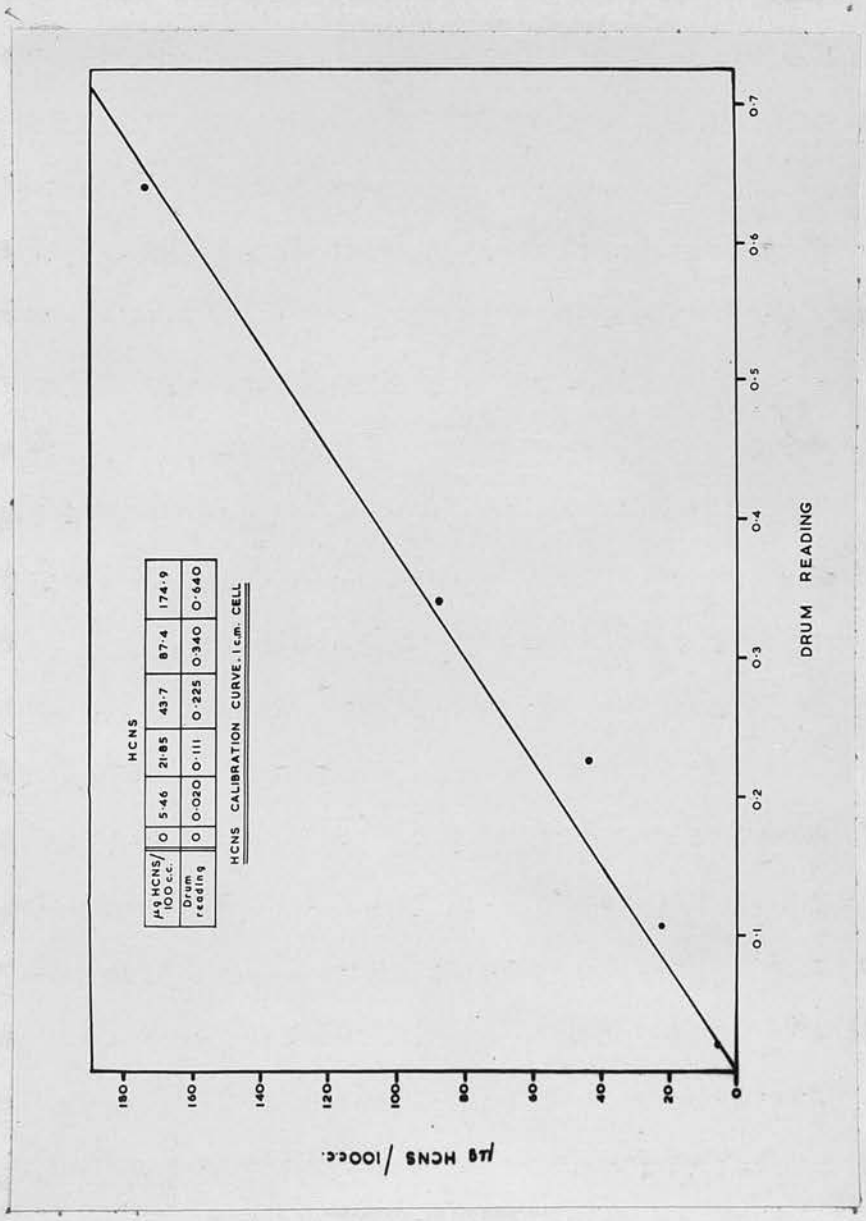


Fig. 25 - HCNS calibration curve.

For deprotenisation 10 cc of 5% trichloroacetic acid are added to 5 cc of the plasma or serum and the mixture well shaken, and filtered through a 9c.m. filter paper (Whatman No.2). The clear filtrate is then used for analysis. The combined HCN and HCNS may first be determined directly after acidifying with a few drops of acetic acid. Cyanides are then removed by bubbling air, saturated with water vapour through the acid solution and thiocyanates left unchanged are measured in a second determination.

Postmortem samples.

50 g. fresh material are homogenized with 100 cc water, transferred to a 500 cc Kjeldahl flask and treated in the manner described for bracken.

V Tests applied to procedures used

To study the accuracy of the procedures used in the determination of HCNa number of tests were carried out with bracken, urine and blood using both the alkaline titration and the benzidine methods of determination.

The effect of time of maceration on the HCN obtained from bracken

Two samples were weighed from each of two types of bracken, homogenized, and macerated with the addition of tartaric acid. One of each pair of samples was aerated after 24 hours while the other

was kept in a refrigerator for two weeks before aeration. The results given below show only a slight loss of HCN resulting from the prolonged period of maceration at low temperature.

mg. HCN/100 g D.M. (determined by benzidine method)

Sample	Time of maceration 24 hours	2 weeks
Young bracken shoots	13.18	12.60
Mature bracken	0.248	0.227

some of the experimental samples of bracken were macerated for periods up to one week, but in view of the results of this test it may be concluded that the figures obtained would be quite reliable.

The effect of adding emulsin during maceration.

mg HCN/100 g. D.M.

Sample	Emulsin Added 0.2g.	Nil
Dried bracken	1.978	2.108
Dried bracken leaf	3.038	2.976
Whole bracken cubes	2.294	1.922
Alcohol extract of bracken	1.798	1.674

These figures show that with most bracken materials the addition of 0.2g emulsin during maceration did not increase the HCN yield.

The effect of tartaric acid on the recovery of HCN and a comparison of the aeration and steam distillation methods

When tartaric acid was added recovery of HCN from an aqueous solution of NaCN was 98.15% by the aeration method and 98.11% by the steam distillation method. Without tartaric acid, however the steam distillation method gave a higher recovery than the aeration method but both were low.

	Recovery of HCN			
	with tartaric acid		without tartaric acid	
mg HCN	aeration	steam distillation	aeration	steam distillation
added	7.560	7.560	7.020	7.020
recovered	7.419	7.417	4.782	5.728
% recovery	98.15	98.11	68.12	81.60

These results show the importance of acidifying the sample before aeration and when acidification is employed, show no difference between the steam distillation and aeration methods. The following results were also obtained using three different quantities of NaCN and show little difference between the two methods.

(see over for tables)

Recovery of HCN

Method	mg HCN	A	mg. B	C
Aeration	added	7.560	2.808	1.451
	recovered	7.374	2.633	1.451
	% recovery	97.54	93.90	100
steam distill-ation.	added	7.560	2.808	1.451
	recovered	7.308	2.687	1.472
	% recovery	96.66	95.60	101.5

It is , however, advantageous to use the aeration method since this gives the HCN in a small volume and so facilitates accurate determinations.

The recovery of HCN added to bracken.

	Aeration	steam distillation A	B
HCN in bracken	2.197	2.303	2.250
HCN added	7.010	7.010	7.010
Total HCN present	9.207	9.313	9.260
" " recovered	9.053	9.079	9.063
recovery of added HCN	6.856	6.776	6.813
% recovery	97.80	96.67	97.19

The figures in this table show that HCN in the form of NaCN solution added to the bracken gave a good recovery both by aeration and steam distillation.

The recovery of HCN and HCNS added to urine

	Aeration	steam distillation A	B
HCN in urine	0.212	0.212	0.212
HCN added	7.010	7.010	7.010
Total HCN recovered	6.962	6.856	6.776
recovery of added HCN	6.750	6.644	6.564
% recovery	96.29	94.78	94.21

The recovery of NaCN added to urine, as shown in the above table, was slightly lower than the recovery

from bracken but still within the normal range of experimental error, The recovery of KCNS added to urine as shown in the following table was similar.

	Aeration	Steam distillation
HCNS in urine	1.560	1.560
HCNS added	5.465	5.465
Total HCNS present	7.025	7.025
recovery of HCNS added	5.236	5.263
% recovery	95.80	96.20

The effect of storage of plasma and serum on HCN content

Sample	Drum reading 9.10.48	26.10.48
Plasma	0.218	0.209
Serum	0.182	0.180

Normal bullock plasma and serum containing added NaCN gave in the benzidine method of analysis drum readings as shown in the above table. Storage in the refrigerator for 17 days did not produce any significant change.

2. Results and discussion

A. The HCN content of bracken

In order to obtain information on the effect of stage of growth on HCN content, two types of bracken were sampled on Carnethy hill in August, 1949, viz:- mature green bracken (27% dry matter) and young bracken shoots containing 2 to 3 leaves (21.5% dry matter).

HCN was determined in the whole plant in each case, and also in leaves and stem separately. The figures given in the table over-leaf indicate that the leaves contain 3 to 4 times as much HCN as the stem, and the young plants are about ten times as rich in this constituent as the mature ones. Very young shoots with no leaves unfolded (Fig.1) have been found to contain as much as 43 mg HCN/100 g. dry matter.

That daily and seasonal variations in the HCN content of bracken could be considerable was found when samples were analysed regularly throughout the period from June 18th 1949 to October 22nd 1949. In the first fresh bracken feeding experiment lasting from June 18th to July 16th, 1949 HCN was determined daily in the freshly cut bracken with the results shown in appendix 5 and Fig. 26.

It will be seen that day to day variations were very considerable and there was a definite tendency for the values to decline as the season advanced and the plant matured, although the last three samples showed a marked rise. As the bracken was cut in bulk for feeding to the experimental animals no attempt was made to take samples from randomised areas or to secure bracken of comparable maturity each day, the bracken cut being merely, that most accessible for scything. It may be, therefore, that the bracken

HCN in fresh bracken.

Type of bracken	HCN (mg/100g. D. M. whole plant	leaf	stem	Proportion of whole plant leaf	stem	Dry matter of whole plant.
				%	%	%
Mature green	1.566	1.800	0.696	60	40	27.0
Young shoots 2 - 3 leaves	15.722	20.418	5.222	57.7	42.3	21.5

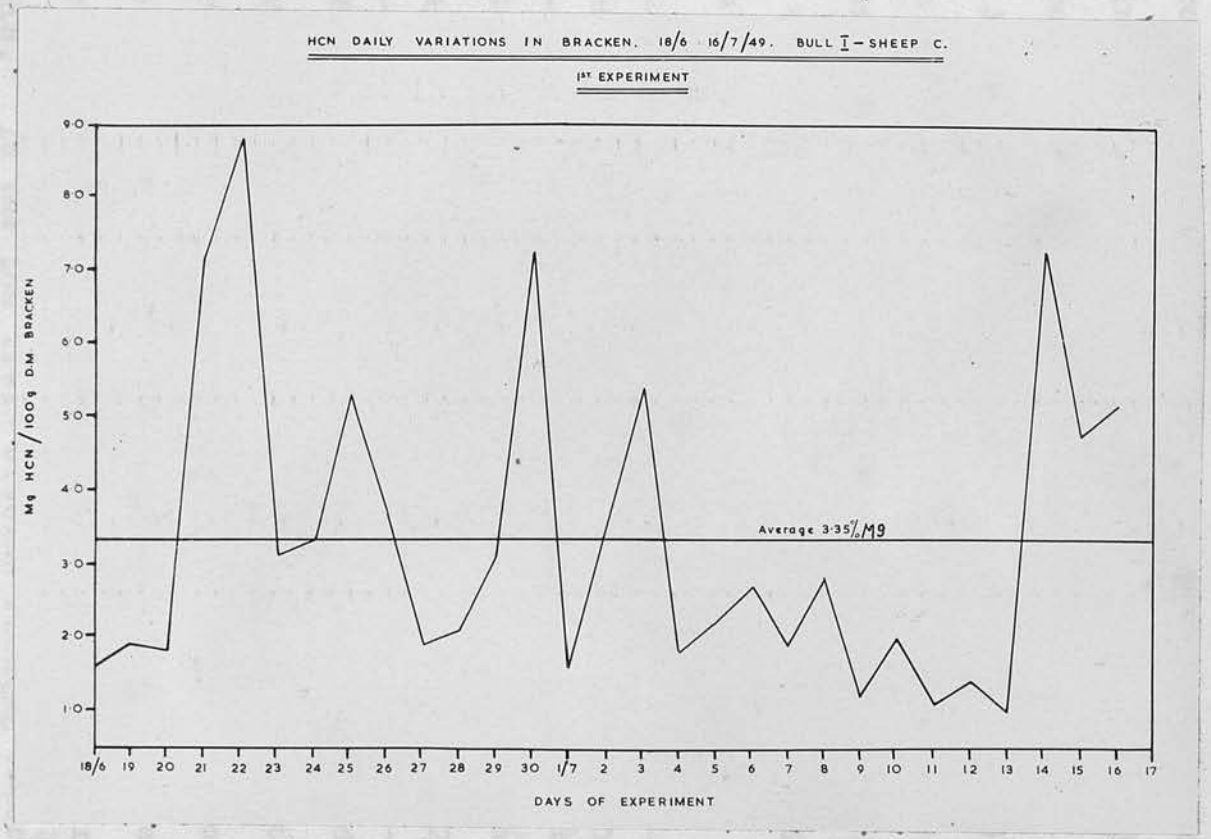


Fig. 26 - HCN in fresh bracken (June-July).

cut on the last 3 days of the first experiment was younger than that cut previously, and was for that reason richer in HCN. The concentration of HCN during this experiment varied between 0.97 and 8.82 mg per 100 g dry matter ~~and~~, with an average of 3.35 mg/ 100 g. dry matter. In the period between July 17th and August 17th when no fresh bracken feeding experiments were in progress samples were collected for analysis on two occasions with the following results.

Type of bracken	HCN in fresh bracken (mg/100g.D.M)	
	28/7/49	11/8/49
Mature green	0.248	1.566
Young shoots	13.18	15.722

The two samples of mature bracken differed widely in their HCN contents but were both well below the average for the preceding period. Young bracken shoots sampled on the same two dates however, were very similar and contained a far higher concentration of HCN. In the 2nd fresh bracken feeding experiment which lasted from August 18th to October 21st, 1949 samples were again analysed daily, with the results given in appendix 5 and Fig. 27 . This experiment was in two very distinct periods; mature bracken rapidly turning brown was sampled between August 18th

and October 8th, whilst a young green second growth was sampled thereafter. The mature bracken again showed day to day fluctuations but these were not nearly so pronounced as in the first experiment. The average content of HCN was much lower than in the first experiment but the tendency for the values to decline as the season advanced was still quite apparent. In the last days of this period the HCN had almost completely disappeared. The young second growth of bracken sampled from October 9th to October 21st 1949, showed a high HCN content averaging 7.35 mg% which was considerably above the average for June-July bracken in the first experiment. The daily fluctuations were again quite marked.

It is clear that the HCN content of bracken fluctuates widely from day to day, the fluctuation being greatest when the average content of HCN is high. The season or maturity factor is also quite well marked, bracken starting at the beginning of the season with a high HCN content and this decreasing gradually as the season advances and the plant matures. Old brown bracken is practically free from HCN. The most important factor influencing the HCN content of bracken is the stage of growth.

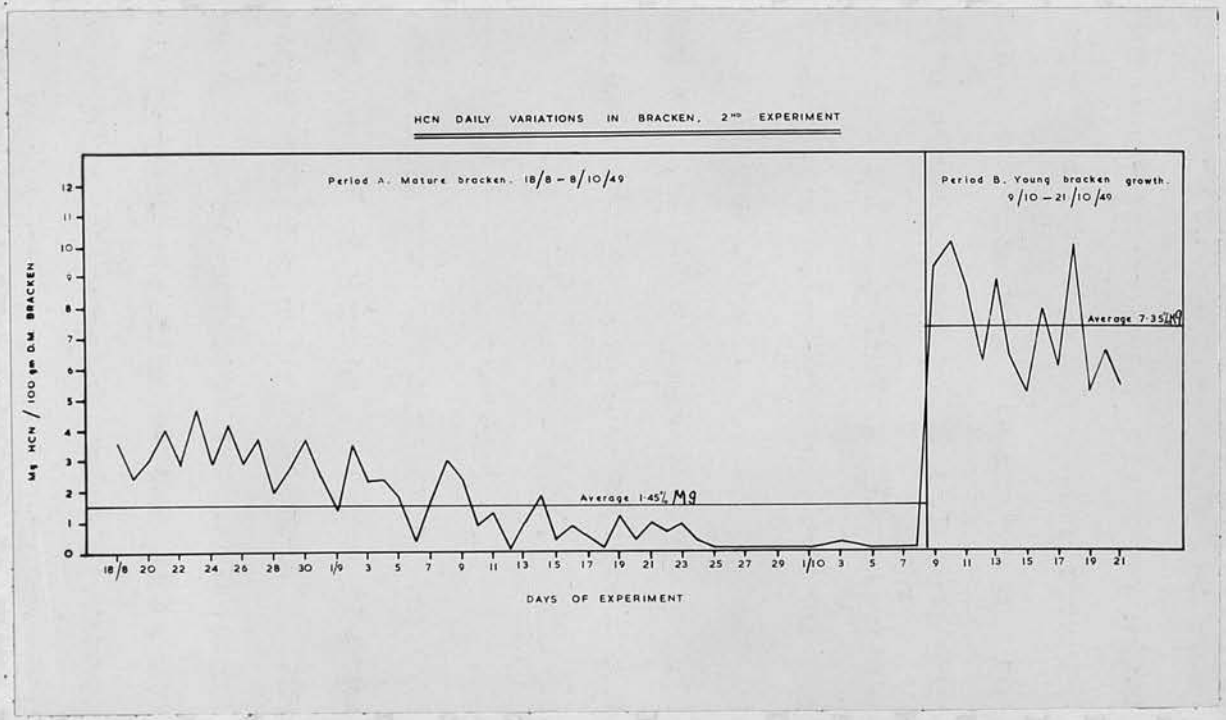


Fig. 27 - HCN in fresh bracken (August-October).

B. The HCN content of artificially dried bracken - and the alcohol extraction of the HCN fraction

Bracken cut on June 30th, 1949 and artificially dried in a commercial grass drier, was found after several months storage to contain 2.07 mg HCN per 100 g. dry matter, whilst sweepings from the floor where this material was stored, consisting largely of bracken leaf material, contained 3.01 mg HCN per 100 g. dry matter. At the time the bracken was cut the fresh material was fluctuating widely in HCN content, between 1.5 and 7.5 mg per 100 g. dry matter. A portion of this dried bracken was hammer milled and cubed in a commercial plant and the cubes were found to contain 2.03 mg HCN per 100 g. dry matter, there being apparently little loss in the cubing process.

Since glucosides have been successfully extracted with alcohol by Finnemore et al (20), Melville et al (40) and many other workers, attempts were made to concentrate the HCN fraction of bracken by alcohol extraction, on the assumption that it is present in the form of a glucoside. Laboratory trials were made using the bracken sweepings referred to above and containing 3 mg HCN per 100 g. dry matter. The product obtained by 8 to 10 hours Soxhlet extraction with 96% alcohol was found to contain the equivalent of 3.97mg HCN per 100 g. of the original dry matter, whilst the

residue after extraction contained only 0.10 mg HCN per 100 g. original dry matter. Since it appeared possible to concentrate the cyanogenetic factor of dried bracken by alcohol extraction 2 to 3 cwt of the dried bracken were hammer milled and alcohol extracted in a commercial plant. The residual bracken meal from this process was thereafter cubed. Analysis showed the extracted bracken cubes to be very low in HCN, as expected, containing 0.155 mg per 100 g. dry matter. The commercial extract, however, was disappointingly low in HCN containing only 1.61 mg per 100 g. dry matter, there being apparently a considerable loss in the extraction process. Since the commercial extraction plant was made of copper this was suspected of forming a complex with the HCN fraction, rendering it inactive and unavailable. Tests made with copper sulphate and sodium cyanide solutions showed a high degree of inactivation as follows.

volume of $CuSO_4$ * solution	mg HCN added	mg HCN recovered	% recovery
8 cc	43.875	17.05	38.86
0.8 cc	43.875	10.075	22.96

* 159g copper sulphate per 1000 cc water.

Tests were then carried out with a laboratory

alcohol extract and copper foil and with the water soluble fraction of the alcohol extract, as shown in the table below.

Material and treatment	HCN mg/100g D.M.	% Recovery
50 cc alcohol extract	2.273	-
50 cc alcohol extract boiled 3 hours with copper foil	2.170	95.47
50 cc alcohol extract evaporated to dryness and extracted with water	2.170	95.47
50 cc alcohol extract evaporated to dryness, water extracted and the extract boiled 3 hours with copper foil.	1.446	66.63

Boiling this alcohol extract for 3 hours with copper foil had little effect and evaporation to dryness and extraction with water also produced little loss. When the water soluble fraction was boiled with copper foil, however, there was a loss of one third of the HCN fraction so that it appears the copper of the extraction plant may have played an important part in lowering the available HCN. Whereas the laboratory tests were carried on for only 3 hours the commercial extraction process involved contact with copper for more than a week.

C. The effect of starvation on urinary HCN excretion

To study the effect of starvation on urinary HCN excretion normal urine samples were first collected from bullock 5 for three days when hay was fed ad lib. The animal was then given only water for the next four days, a small feed of hay (10 lb daily) for the following three days and finally hay ad lib. The daily urines were analysed for HCN and HCNS, the combined figures (as HCN) being shown as total HCN in the table overleaf. The detailed figures are given in appendix 6 .

The HCN in the average normal urine collected before starvation was 0.749 mg /100cc equivalent to an excretion of 25.18 mg HCN per day. During the 4 days starvation these values were markedly reduced the concentration falling to about one fifth and the daily excretion to little more than a third. Nevertheless there was still some excretion of HCN in the urine even after 4 days starvation, indicating that urinary HCN cannot entirely be attributed to the intake in the food consumed. Probably protein catabolism may lead to the production of some of the HCN excreted in the urine. When the feeding of hay was recommenced the urinary HCN excretion began to increase but in 11 days it did not return to the pre-starvation level. The total daily excretion of

Urinary excretion of total HCN (Free HCN and HCNS)

Date of Collection	mg total HCN per day	mg total HCN per 100cc urine
	Full Feed	
10/2/50	21.334	0.757
11/2/50	26.167	0.800
12/2/50	28.045	0.689
Mean value =	25.182	0.749
	Starvation	
13/2/50	17.371	0.345
14/2/50	15.850	0.261
15/2/50	12.665	0.201
16/2/50	9.213	0.169
	Small feed	
17/2/50	2.306	0.193
18/2/50	3.006	0.251
19/2/50	3.960	0.335
	Full feed	
20/2/50	3.184	0.252
21/2/50	3.974	0.239
22/2/50	3.152	0.191
23/2/50	2.011	0.133
24/2/50	3.849	0.332
25/2/50	6.239	0.378
26/2/50	7.631	0.332
27/2/50	7.015	0.385

HCN continued to fall even when a small feed of hay was given and it took 6 days of feeding on hay ad lib to produce any recovery at all. 11 days after the end of the starvation period the total daily excretion of HCN was still below the level at the time of starvation and much below the level found before starvation. The urinary excretion of HCN would appear to be influenced by several complex factors but without a very extended investigation there is little prospect of elucidating these factors. It is clear that urinary HCN figures need to be interpreted with considerable circumspection.

D. The apparent HCN balance in fresh bracken feeding experiments.

During the fresh bracken feeding experiments, HCN was determined daily in the bracken fed and the total HCN consumed by each animal was calculated. The HCN and HCNS in the daily urine samples were also determined and the total HCN excretion obtained. Tests showed no detectable HCN in the faeces. The apparent cyanide balance for each animal was then calculated, with the results shown in the following table, and appendix 2 (page 7).

It appears that the two bullocks were able to excrete through the urine about 60% of the HCN consumed in the bracken. If the animals were unable to detoxicate more than the amounts excreted in the urine

Apparent HCN balances for animals fed on fresh bracken

Animal	Period of Experiment	No.of days.	HCN	HCN (g) consumed	HCN excreted in urine (g)	Apparent HCN (g) balance	HCN retention %
Bullock No.1	18/6/49-17/7/49	29	Total	3.6435	2.2691	1.3744	37.76
			aver./day	0.1256	0.0782	0.0474	
Bullock No.4	18/8/49-24/10/49	65	Total	2.2333	1.3767	0.8566	38.41
			aver./day	0.0344	0.0212	0.0132	
Bullock No.4	9/10/49-24/10/49 (2nd growth only)	13	Total	1.2493	0.4039	0.8454	67.67
			aver./day	0/0961	0.0311	0.0650	
Sheep E	18/8/49-21/10/49	65	Total	0.2914	0.0652	0.2262	77.62
			aver./day	0.0045	0.0001	0.0035	
Sheep F	18/8/49-23/10/49	66	Total	0.7639	0.2376	0.5263	68.86
			aver./day	0.0116	0.0036	0.0080	

a considerable proportion of the HCN must have been retained in the body. With bullock 4 the daily intake in the first 53 days of the experiment, whilst mature brown bracken was fed, was extremely low and on some days was negligible (Fig. 27 page 131). For this reason a separate balance was calculated for the last 13 days of the experiment when the young second growth of bracken was fed. In this period the apparent retention increased to 68.0% as was expected; the average daily intake of HCN also increased to 0.0961 g which is much nearer to the average daily intake of bullock 1.

Although sheep E which did not die from bracken poisoning showed an apparently high percentage retention of the bracken HCN consumed, the absolute retention (0.226g.) was small, owing to the very low food consumption. Sheep F on the other hand which did die from bracken poisoning showed a lower percentage retention than sheep E but an absolute retention about 2½ times as great as sheep E. The results with these sheep therefore do not rule out the possibility of the HCN fraction of bracken being involved in the production of bracken poisoning. If the quantities of HCN apparently stored by these experimental animals are compared with the toxic doses of HCN quoted by several investigators, it

seems that they would be extremely fatal if consumed in a single dose. Steyn (58) and Couch etal (14) reported that the lethal dose of HCN for an animal is roughly 1 mg HCN per lb live weight, so that for a bullock weighing approximately 700 lb the lethal dose is in the region of 0.7g. HCN and for a sheep of 100 lb it will be in the region of 0.1g. Thus the amounts of HCN present in bracken and apparently retained to an appreciable extent in the animal body may play an important part in the production of bracken poisoning.

It should also be noted that in the second experiment the three animals were fed on mature bracken low in HCN, for 53 days without any poisonous effect. When, however the second growth of young bracken, relatively rich in HCN was fed, Bullock 4, and sheep F developed bracken poisoning in 12 days. Thus the poisoning of animals fed on bracken is closely related to the stage of growth of the plant which in turn is closely related to the HCN content. There are however two aspects of cyanide metabolism which may be of considerable importance and which could not be satisfactorily dealt with in the present work. One is the urinary excretion of HCN and HCNS not derived from dietary HCN; the difficulty of making any proper allowance for this fraction was shown in the

examination of the effect of starvation on urinary HCN (page 136). The other is the respiratory excretion of HCN in gaseous form, which could not be measured in the present work, although tests with sodium picrate paper indicated some excretion by that route.

The failure to promote recovery in bullock 1 (first experiment) by injecting sodium thiosulphate and sodium nitrite as suggested by Steyn (58), Couch et al (14), Schubel (53), Chen et al (10) and Van Der Walt (66), for the treatment of HCN poisoning, may be due to the fact that poisoning was detected only 48 hours before death and treatment applied less than 24 hours before death. At that stage extensive and irreversible changes may have occurred internally.

HCN in blood and postmortem samples

Figures for HCN and HCNS in plasma and serum are given in appendix 7 and summarised in the table overleaf.

These figures show that there were no very big increases in the HCN content of plasma and serum after the feeding of bracken but quite marked increases in HCNS content were observed in most cases. These increases were to be expected, being the result of the normal HCN detoxication process.

HCN and HCNS in blood of animals fed on fresh bracken

Experiment	Animal	Plasma				Serum			
		mg HCN/100cc		mg HCNS/100cc		mg HCN/100cc		mg HCNS/100cc	
		Hay fed	Bracken fed	hay fed	bracken fed	hay fed	bracken fed	hay fed	bracken fed
1st	Bullock 1	0.003	0.008	0.087	0.525	0.006	0.013	0.037	0.422
	Sheep C	0.003	0.012	0.087	0.169	0.003	0.008	0.124	0.161
2nd	Bullock 4	Nil	0.007	0.273	0.431	-	0.008	-	0.463
	Sheep E	Nil	0.003	0.093	0.098	0.006	0.006	0.174	0.093
	Sheep F	Nil	0.004	0.093	0.156	0.003	0.003	0.106	0.206
Control data for hay fed animals.	Sheep C	0.001	-	0.062	-	0.003	-	0.149	-
	Sheep D	0.005	-	0.077	-	0.006	-	0.099	-

The HCNS in the plasma of sheep E, however did not increase significantly whilst the serum content showed a decline. This sheep, as noted previously, showed effects due to starvation and did not die from bracken poisoning.

Postmortem samples of liver and of rumen and abomasum contents from the experimental animals were also analysed for HCN with the following results.

HCN in postmortem samples (mg/100g. fresh material)

Animal	Liver	Rumen content	Abomasum content
Bullock 1	Nil	0.025	Nil
" 4	0.012	0.050	0.025
Sheep E	Nil	0.025	0.012
" F	0.025	0.037	0.012

Van Der Walt (66) has reported the presence of small amounts of HCN in normal rumen contents and the amounts shown in the above table cannot be regarded as of any significance. Similarly the values for abomasum contents and for liver samples do not provide any evidence of death from HCN poisoning.

E. The apparent HCN balance in dried bracken feeding experiments.

During the feeding of chopped dried bracken, whole bracken cubes and extracted bracken cubes to the sheep C, D and G, the urinary HCN and HCNS excretion

was determined. The values are given in appendix 3 and the figures for total urinary HCN (i.e. HCN and HCNS) are summarised below, from appendix 6 (page 50).

Sheep	Feed	No. of days	Total HCN av. per day	Excretion (mg) av. per 100 cc
G*	hay	3	1.324	0.183
	dried bracken	70	3.080	0.260
D*	hay	3	2.831	0.364
	dried bracken	56	1.044	0.160
	whole bracken cubes	44	2.635	0.173
C	hay	2	2.553	0.408
	dried bracken	38	2.263	0.225
	extracted bracken cubes	44	5.310	0.170

* These two sheep developed bracken poisoning and died.

The concentration of total HCN in the urine of sheep G increased by over 40% when dried bracken was fed and the average daily excretion increased by over 130%. In the case of sheep D, however, both the urinary concentration and the daily excretion of total HCN decreased when bracken was fed. Sheep C also showed a lowered concentration during bracken feeding but a marked rise in total excretion when extracted bracken cubes were fed, despite the very low HCN content of these cubes. The extracted cubes were, however, very palatable and large quantities were consumed and large volumes of urine produced in this period.

Apparent HCN balances calculated for these three sheep are shown on page 146.

The apparent percentage retention of HCN by these animals was high in all cases except for sheep C when extracted bracken cubes were fed and the excretion of HCN exceeded the intake by about 80%. In this period the HCN in the food consumed was low and there was a very large volume of urine excreted so that the normal urinary HCN not derived from dietary HCN may have formed a large proportion of the total. As stated previously it was not possible to attempt any accurate correction for this factor.

The overall HCN balances for sheep G, D. and C. shown in the table on page 146, are greater than the balance (+0.53g) found for sheep F, which died of bracken poisoning when fed on fresh bracken (see p. 138). Sheep G, with an apparent balance of +0.64g. was comparable with sheep F but sheep C did not develop bracken poisoning at all, even though it appeared to retain 1.1g HCN, more than twice the balance of sheep F, whilst sheep D did not develop bracken poisoning until it had retained 1.5g., which is nearly three times the retention of sheep F. There is clearly no close relationship between apparent HCN balance and the incidence of bracken poisoning, but some uncertainty must be attached to the balance

Apparent HCN balances for animals fed on dried bracken

Sheep	Period	No. of days	Feed	Total quantity consumed Kg	mg HCN/100g D.M.	Total HCN consumed g.	HCN excreted in urine per day mg	HCN excreted in urine in total period g.	HCN balance	% HCN retention
G	16/3/50-24/5/50	70	dried bracken	42.841	2.071	0.860	3.080	0.216	+0.644	75
D	16/3/50-10/5/50	56	dried bracken	25.804	2.071	0.534	1.044	0.058	+0.476	90
	11/5/50-23/6/50	44	whole bracken cubes	39.924	2.025	0.808	2.635	0.116	+0.692	80
	24/6/50-9/7/50	16	dried bracken	17.097	2.071	0.354	1.044	0.017	+0.337	95
	Total	186	-	-	-	1.696	-	0.191	+1.505	90
C	3/4/50-10/5/50	38	dried bracken	20.522	2.071	0.425	2.263	0.085	+0.340	80
	11/5/50-23/6/50	44	extracted bracken cubes	85.935	0.155	0.133	5.310	0.234	-0.101	-80
	24/6/50-28/6/50	5	dried bracken	5.075	2.071	0.105	2.263	0.011	+0.094	90
	29/6/50-8/7/50	10	whole bracken cubes	17.271	2.025	0.350	2.263	0.023	+0.327	79
	9/7/50-31/7/50	23	dried bracken	24.936	2.071	0.516	2.263	0.052	+0.464	90
	Total	120	-	-	-	1.529	-	0.405	+1.124	73

figures because of the impossibility of making any valid correction for urinary HCN not of dietary origin and because of the absence of quantitative information regarding the respiratory excretion of this poison. It should be noted, however, that in their effects on the HCN balance data these two factors act in opposite directions and so may to some extent compensate one another.

Values for the HCN and HCNS in the plasma of sheep C, D and G are given below and show that dried bracken feeding had little effect on HCN and in some cases only a small effect on HCNS.

Sheep	Feed	Blood plasma HCN/100cc	HCNS/100cc
G	hay fed	Nil	0.050
	dried bracken	Nil	0.050
D	hay	0.019	0.025
	dried bracken	Nil	0.087
	whole bracken cubes	0.025	0.198
C	hay	0.003	0.053
	extracted bracken cubes	0.012	0.149

F. The feeding of a commercial alcohol extract of bracken.

During the feeding of the alcohol extract along with hay to bullock 5 urine samples were collected for total HCN determination, with the results shown in appendix 4-6 and the following table.

Date	Feed	mg HCN excreted per day	per 100cc urine
25 - 26/6	hay	6.897	0.243
27/6-1/7	alcohol extract	9.733	0.230
2/7 - 6/7	" "	12.421	0.230
7/7-10/7	" "	9.399	0.244
Mean value	" "	10.518	0.235

There was no significant change in the HCN concentration of the urine and only a relatively small increase inthe daily excretion. This is not surprising in view of the relatively small amount of HCN which found to be recoverable from the commercial extract by the aeration method.

G. The feeding of HCN

The NaCN feeding experiments were carried out with bullock 5 and sheep H. Bullock 5 consumed in 59 days a total amount of NaCN equivalent to 6.657g. HCN and sheep H consumed NaCN equivalent to 4.220 g. HCN in 78 days. There was no poisonous effect in either case. Urine samples were collected for HCN deter-

mination during this experiment and the figures obtained are given in appendix 4+6and summarised in the following table.

Bullock 5 Total HCN in urine

Period of Collection	average HCN excreted (mg.) per day	per 100 cc
	Hay fed only	
13/3/50	11.315	0.477
15/3/50	14.638	0.552
Mean value	12.977	0.515
	Fed NaCN≡ 89.35 mg HCN per day (+hay)	
16-/9-3-50	14.036	0.513
24-27/3	16.292	0.534
1 - 4/4	14.817	0.549
9 - 12/4	10.900	0.409
Mean value	14.011	0.501
	Fed NaCN≡ 134.03 mg HCN per day(+hay)	
14-19/4	12.885	0.489
21-26/4	10.959	0.435
28-3/5	11.237	0.430
6 - 9/5	10.258	0.372
10/5	9.631	0.327
11/5	8.665	0.315
12/5	11.912	0.404
Mean value	10.792	0.396

Sheep H Total HCN in urine

Period of Collection	Average HCN excreted (mg) per day	per 100 cc
	Fed hay only	
14/3/50	1.857	0.262
15/3	2.496	0.347
14/4-19/4	2.997	0.410
13 - 15/5	2.382	0.360
Mean value	2.433	0.345
Drenched with NaCN≡ 54.108mg HCN per day(hay fed)		
16/5	2.848	0.407
17-28/5	1.365	0.210
29-31/5	2.868	0.370
2 -7/6	2.271	0.290
Mean value	2.338	0.319

These figures show no increase in either the concentration or the total quantity of HCN in the urine although the HCN was administered with the food in one case and as a drench in the other.

Tocher (61) in 1935 found that when KCN solution was mixed with food and offered to a pig it was refused for several hours by which time volatilisation with the production of formic acid was probably complete and there was no poisonous effect. In another experiment Tocher was able to cause death in 30 minutes with the same animal following administration of the same quantity of KCN but in capsule form.

Changes of a similar sort may have occurred in the experiment with the bullock, whilst the dried grass cubes containing the HCN were in the rumen.

This is supported by the fact that there was no increase in urinary HCNS excretion (see appendix 6 page 54) as would be expected if the HCN was absorbed into the body without change.

In the case of sheep H it was hoped that administration by drench would lead to passage of NaCN solution directly to the omasum, thereby reaching the abomasum relatively rapidly. Whether or not this occurred, could not be ascertained but as the urinary excretion of total HCN showed no increase it appears that in this case also decomposition of the cyanide occurred before absorption.

The absence of increased urinary excretion of total HCN following administration of NaCN indicates that the NaCN feeding experiment is not comparable with the experiment in which bracken was fed and the absence of any symptoms following the feeding of NaCN does not necessarily indicate that the cyanide factor in bracken has no effect on the animal. In the case of bracken the several hours maceration in the rumen may be necessary for the release of the HCN, whilst in the case of NaCN the period in the rumen may be responsible for the complete destruction of the toxic material. Since the excretion of HCNS increased following bracken feeding it is clear that HCN was absorbed into the system although of course, there is

no certainty that absorption was complete; there may have been partial destruction and partial absorption and if that were the case the apparent HCN balance figures obtained would be too high.

When fed on hay, bullock 5 plasma was found to contain 0.006 and 0.124 mg per 100 cc of HCN and HCNS respectively. When given NaCN the corresponding values obtained were 0.012 and 0.332 so it appears that at the time of sampling there had probably been some absorption and detoxication of HCN although the urine figures indicate that this was probably negligible.

Summary

1. Bracken at the beginning of the season has a high HCN content which decreases gradually as the plant matures towards the end of the season.

2. The HCN content of bracken depends primarily on the stage of growth whatever the season.

3. It appears that the HCN fraction of bracken may play some part in the production of bracken poisoning but the evidence is far from conclusive.

4. The poisoning of animals fed on fresh bracken is closely related to the age of the bracken which in turn is closely related to the content of HCN.

5. Fresh bracken feeding produced deaths with typical bracken poisoning symptoms, and the apparent HCN

balances equalled or exceeded the lethal level.

6. Extracted bracken cubes contained very little HCN and even when consumed in large amounts did not produce any poisoning.

7. A commercial alcohol extract of bracken appeared to have suffered inactivation or destruction of the HCN-containing substance and did not produce any poisoning when fed to a bullock.

8. Feeding quantities of NaCN providing amounts of HCN equivalent to or greater than those found in bracken failed to produce poisoning but the analyses indicate that the two experiments were not comparable and that when NaCN was fed, destruction before absorption was complete or almost so.

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PART IV

The rôle of vitamin B_1 in relation to bracken poisoning

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Introduction

Recent work by Weswig, Freed, and Haag (44) in 1946 and by Thomas and Walker (39) in 1949 has indicated that for rats, bracken is toxic because it induces thiamin deficiency. Rats on an adequate thiamin diet containing 40% bracken, lost weight and died within 30days, whereas control animals receiving large supplements of thiamin exhibited normal growth rates.

These results with rats and recent results by Roberts et al (35) with horses, cannot with any certainty be applied to cattle and sheep, although they do suggest that bracken poisoning in farm livestock may be due to an induced thiamin deficiency.

In order to throw some light on the significance of vitamin B_1 in bracken poisoning in ruminants various assays of this vitamin were made in the bracken feeding experiments carried out with bullocks and sheep. Vitamin B_1 was determined in urine samples specially collected at various times during each experiment, as well as in postmortem tissue samples from the experimental cases of bracken poisoning.

Attempts to determine the vitamin B_1 in blood were abandoned when the results were found to be unreliable,

owing to the very low concentration of this vitamin, even in normal blood.

Since vitamin B_1 is necessary for the oxidation of pyruvic acid as mentioned by Peters (30), vitamin B_1 deficiency would be expected to influence the pyruvic acid metabolism in the animal body, and to produce an increased level of pyruvic acid in blood and urine. For that reason determinations were made of the pyruvic acid in blood and urine samples collected from both normal and brecken fed animals.

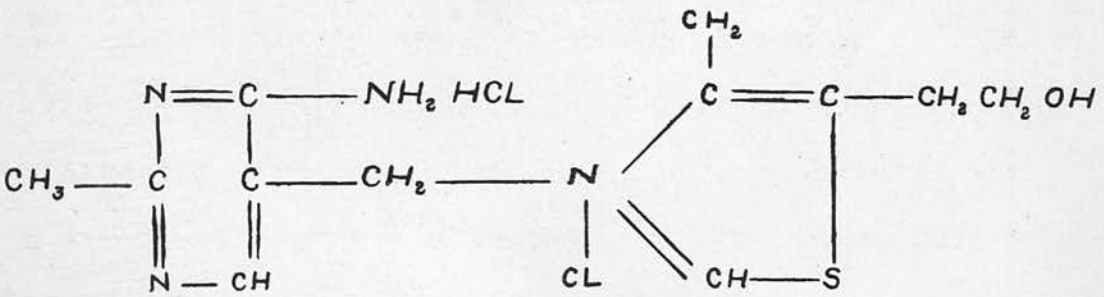
The alkalinity of the urine samples was also measured in order to see whether the degree of alkalinity was influenced by feeding bracken, since the alkalinity factor alone, might be responsible for the destruction of the vitamin B_1 of the urine, whilst this was still in the bladder.

In some experiments animals showing symptoms of bracken poisoning were given injections of vitamin B_1 but this treatment did not prevent death.

Review of literature

Vitamin B_1 called aneurin or thiamin, is required in the metabolism of both plants and animals. The higher plants synthesise it and so do many but not all of the lower forms; Abdel-salaam and Leong (1) have reported that some types of bacteria produce vitamin B_1 whereas others do not. All animals, however must

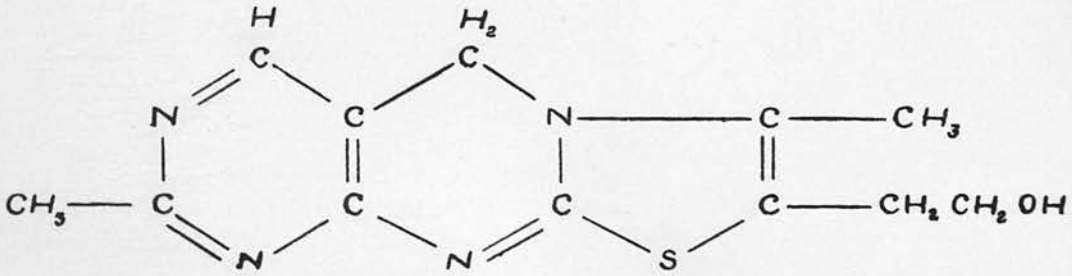
have a dietary source of this vitamin, unless it is synthesised for them by micro-organisms in the digestive tract, as is the case with ruminants. Characteristic signs of vitamin B_1 deficiency are loss of appetite and weight. In advanced stages there are increased concentrations of pyruvate and lactate in the blood, and polyneuritis may occur. Vitamin B_1 has the following structural formula.



THIAMIN HYDROCHLORIDE

Fig. 28.

In neutral or alkaline solutions it is rapidly destroyed, presumably because of decomposition of the thiazole portion. At pH 3.5, however the vitamin will withstand sterilization for half an hour at 120°C without loss of activity. Sulfite will cause a cleavage of the molecule in solutions of pH 4 - 6, whilst "in vitro" oxidation of the vitamin yields thiochrome which is biologically inactive.



THIO CHROME

Fig. 29.

Carbohydrate metabolism in vitamin B_1 deficiency.

Until recently little attention has been paid to the occurrence in body fluids of carbonyl compounds other than the ketone bodies. Pyruvates do not appear to be toxic in the amounts occurring in vitamin B_1 deficiency.

The accumulation of pyruvic acid in the blood in vitamin B_1 deficiency was first shown by Thompson and Johnson (40) in England, using rats and pigeons, and by Platt and Lu (31) in China, with human beri-beri cases. They were able to relate the increase in bisulphite binding substances to vitamin B_1 deficiency by demonstrating that the raised level returns to normal after administering vitamin B_1. In avitaminosis B_1 the increase is chiefly due to pyruvic acid and keto glutaric acid, but as the bisulphite-binding aldehyde and keto compounds increase in many other diseases it is doubtful whether the measurement of bisulphite-binding substances is a reliable indication of aneurin deficiency.

The inability to oxidise pyruvic acid in the absence of vitamin B_1 has been examined in many species including humans. Platt and Lue (32) in 1936 showed an increase of pyruvic acid in the circulating blood of beri-beri cases, so an increase in blood pyruvate was used as a diagnostic sign of beri-beri. In 1939

Platt and Lu (33) showed that the severity of beri-beri in humans could be correlated with the concentration of pyruvic acid in the blood. Johnson and Edwards (19) and Lu and Platt (27) found an increase of pyruvic acid in the blood and urine of healthy individuals after severe exercise and even moderate muscular exercise will considerably increase the level of blood pyruvate. Furthermore, Platt and Lu (32) could not detect any significant change of blood pyruvate in the atrophic type of beri-beri, which is supposed to be vitamin B_1 deficient. Schroeder (36) in 1939 claimed that a consumption of 250g. of glucose daily by two patients reduced their vitamin B_1 excretion to nil in a fortnight; during this fortnight the vitamin B_1 excretion fluctuated widely.

Himwick and Himwick (17) found that the liver of vitamin B_1 deficient dogs, still continues to extract pyruvate and lactate from the blood and to release its quota of glucose. This observation showed that vitamin B_1 is not necessary for the various steps in the conversion of pyruvic acid to glycogen, but rather for the oxidation of pyruvic acid. The rate of pyruvate usage in pigeon liver was shown by Krebs and Eggleston (22) to depend on the concentration of CO_2 and bicarbonate. This supports the view that

the removal of pyruvate is an active metabolism involving CO_2. Inasuspension of liver from vitamin B_1 dificient pigeons the rate of pyruvate usage is however greatly reduced.

Randoin and Simmonet (34) showed that rats can thrive on a diet free from vitamin B_1 if it contains no carbohydrates. Such animals grow well and do not develop signs of deficiency. Earlier workers also showed that rats can thrive on a diet free from vitamin B_1 if it contains a large proportion of fat or protein. Evans and Lepkovsky (10) in 1928 claimed that rats can be maintained on a low intake of vitamin B_1 provided their ration contains a high proportion of fat and since then several studies have been made of the effect of fat in delaying the onset of Beri-beri and polyneuritis. Benerji (5) found that the vitamin B_1 sparing action of fat is not limited to its effect in protecting such animals against loss of weight or in preventing the development of more marked symptoms of deficiency; even in the absence of these two characteristic signs of avitaminosis B_1 fat has a definite vitamin sparing action.

It now appears that vitamin B_1 functions as the prosthetic group of a respiratory enzyme; by its action, glucose is converted via pyruvate to CO_2 and

water, with the liberation of energy. When vitamin B_1 is absent or insufficient there is an accumulation of pyruvic acid and a failure to obtain the full energy value of ingested carbohydrates. The exact path of conversion of pyruvic acid in human tissues has not been fully confirmed, but the idea that thiamin occurs as the pyrophosphate, which funtions as a co-enzyme in the decarboxylation of pyruvate was proved by Banga et al (3) in 1939.

Pyruvic acid metabolism in normal and in vitamin B_1 deficient humans has been studied by Wortis et al (48), Arakawa (2), Wilkins (45), Platt and Lu (31,32, 33), Friedmann (13), Wilson (47), and Kendall et al (21). Similar work with rats and dogs has been carried out by Shils et al (37), Benerji (5), Li et al (25), Bueding et al (6), Flock et al (11) and Evans et al (9), Pyruvic acid values recorded by these workers are tabulated overleaf.

Pyruvic acid in Blood and Urine

Reference	Animal	Sample	Pyruvic acid (mg/100ml) Normal values	B_1 deficient values
Wortis et al (48)	human	blood	normal-1.44-3.63 (av.217) fasting-0.77-1.17(av.0.98)	-
Arakawa (2)	"	"	- -	0.38 - 0.66 (av. 0.49)
Wilkins et al (45)	"	"	-	0.46 - 0.73
Platt and Lu (33)	"	"	0.40 - 0.75 mg/100g	subacute beri-beri 0.17-1.93(av. 0.93) acute beri-beri 1.0 - 5.77 (av.2.72)
Friedman (13)	"	"	0.77 (3 hours after meal)	-
Wilson (47)	"	"	0.53 - 1.98 (children)	-
Platt and Lu (31,32)	"	Urine	0.25-0.65 (occasionaly 0.7-1.25)	acute Beri-beri 3.7 - 5.33
Kendall (21)	"	"	Nil - 4.5(morning urine)	-

Pyruvic acid in Blood and Urine (cont.)

Reference	Animal	Sample	Pyruvic acid (mg/100ml) Normal values	B_1 deficient values
Shils et al (37)	Rats	urine	0.70 - 3.5	1.10 - 10.0
Benerji (5)	"	"	-	2.0 - 20.0 Bisulpite binding substance calculated as pyruvic acid
Evans et al (9)	"	Blood	0.90	2.8 - 3.9
Li et al (25)	"	"	0.96	after 1 month B_1 deficiency 3.49 during 2nd month deficiency 5.62 (values rose markedly shortly before death)
Bueding et al (6)	Dogs	Blood	0.74 - 1.10	1.72 - 2.90 (after insulin injection)
Flock et al (11)	"	"	1.5 6-16mg (after Pyruvic acid injection) became normal after 3 hours.	

Vitamin B_1 in the normal body

Ochoa and Peters (29) found that the administration of vitamin B_1 to animals leads to an immediate accumulation of both vitamin B_1 and its pyrophosphate in the liver. This brings the liver into prominence in the metabolism of vitamin B_1.

Jolliffe et al (20) using a well controlled deficient diet, measured the excretion of the vitamin before and after oral administration of vitamin B_1. They found that humans showing definite deficiency of the vitamin excreted less than 0.1 mg daily whilst normal subjects excreted over 2 mg daily. In 1940, Wang and Yudkin (42) found a very similar excretion of vitamin B_1 by 3 humans with the same low level of intake. This suggests that the rate of mobilization of the body's stores of vitamin B_1 was similar in the three individuals. With higher intakes the differences in excretion between the three individuals became more noticeable, although their requirements would be of the same order. Possibly the different degrees of excretion represent differences in the rate of absorption and differences in the kidney threshold.

Storage of vitamin B_1 in the body is widespread. Leong (23) in 1937 found that the highest concentration in rats was in the liver and heart. The vitamin B_1 concentration in the muscle is low in the rat, the

young chick, and the ox, but is substantially higher in the adult hog and in the adult pigeon as stated by Williams and Spies (46). Figures obtained for vitamin B_1 in normal and deficient animals have been given by different workers. These are shown in the table overleaf.

Effect of vitamin B_1 deficiency on tissue contents

In 1932, Westenbrink (43) reached the conclusion that, in rats, the liver loses 4/5 of its vitamin B_1 during a single week of deficiency and that the loss then progresses more slowly till only about 1/20 of the original content is left at the end of five weeks of deficiency. The kidney, starting at nearly the same level as the liver, loses at a like rate. The heart, perhaps a little richer in vitamine B_1 than the liver and kidney, loses equally rapidly in the first week, but much less thereafter. Muscle and lungs were found to have lower vitamin B_1 contents and on deficient diets they exhibit more gradual losses. Of all tissues the muscle most nearly reaches complete depletion.

Pigeons lose the greatest part of their vitamin B_1 within 13 days on a deficient diet. In spite of the high level of vitamin B_1 in pigeon muscle, after two weeks on a deficient diet the vitamin B_1 contents

Origin	Animal	Vitamin B_1 (µg per lg. fresh tissue) heart	liver	kidney	muscle
Mickelsen et al (28)	Ox	6.75	2.7-3.9	3.15	0.3-3.0
Mickelsen et al (28)	Ox	1.75	3.80	2.22	-
Harris and Wang (16)	"	-	2.79-3.3	-	-
Mickelsen et al (28)	Sheep	-	4.14	-	3.57
Harris and Wang(16)	"	-	1.95-4.2	-	-
Singher et al (38)	Rat (normal)	-	2 - 16.1	-	-
Greenberg etal (15)		3.7-4.9	4.3	4.1-5.6	0.7-0.8
Singher et al (28)	Rat (B_1 deficient)	-	0.1-2.6	-	-
Greenberg et al (15)		0.5-2.6	1.6	0.5-3.2	0.2-0.3
Leong(23)	Rat	4.2	4.5	1.2	1.2
Leong(24)	Rat(normal)	8.1	7.8	-	1.8
Leong(24)	Guinea-pig	-	2.1	-	0.9
	Fowl	-	2.1	-	0.9
	Pigeon	-	3.3	-	3.6

of rat and pigeon muscle are very similar since in the case of the pigeon there is a more rapid loss at first.

Methods of analysis

A. Vitamin B_1

Methods which have been proposed for the determination of thiamin can be classified into (1) animal, (2) microbiological and (3) chemical or physical.

Animal assays were developed first, following observations of the failure of man or animals to thrive on vitamin B_1 deficient diets.

The microbiological methods include fermentation procedures and methods based on the growth or acid production of bacteria, yeasts, or fungi.

Chemical analyses can be carried out rapidly and are more applicable to routine determinations than some of the other methods. The commonest chemical method involves conversion of vitamin B_1 to thiochrome which may be estimated by measuring the intensity of its fluorescence. This is the method used in the present work. When vitamin B_1 is oxidised (in an alkaline medium it is converted) into thiochrome, as first stated by Barger et al (4) in 1935. This substance has a vivid blue fluorescence which can be detected in dilutions of 1:2,000,000. Recently in 1936, Jansen (18) published a quantitative method for the measurement of vitamin B_1 dependent on the

conversion to thiochrome and the subsequent measurement of fluorescence by means of a photo-electric instrument. This method is suitable for application to a wide range of materials, and the accuracy is high compared with other methods of vitamin B_1 assay; recovery of added vitamin B_1 is usually good.

A difficulty which has been encountered in urines is the presence of various non-specific substances which may seriously interfere in the measurement of the thiochrome fluorescence. To overcome this and other sources of error Wang and Harris (41) in 1939 recommended a number of modifications in the technique; these changes also increased the simplicity and sensitivity of the method. The results proved to be accurate when checked against biological assays, and vitamin B_1 added to urine was quantitatively recovered. Using this method the error is generally less than 10% and a sample of about 5 ml suffices for a determination.

In the present work the method of Wang and Harris (41) was found satisfactory for the determination of vitamin B_1 in animal tissues and urines and details of the procedures followed are given below.

Vitamin B_1 in animal tissues

5g. of fresh tissue are weighed in a basin, and moistened with 3 drops of concentrated hydrochloric

acid, chopped into small pieces with scissors, then transferred to a 100 cc homogenising tube. The basin is rinsed with 30 cc of acetate buffer solution pH 4.45, which is then added to the homogenising vessel. After homogenising for 2 or 3 minutes 0.5 to 1cc iso-butanol is added to dissipate any foam and all the contents are then transferred to a measuring cylinder, rinsing the tube with buffer solution and finally making the volume to 50cc. The solution is well mixed and then transferred to a small conical flask with the addition of 0.2g each of Taka-diastase and papain and 2 to 3 drops of toluene. The flask is stoppered and incubated overnight at 45°C. After incubation the contents of the flask are centrifuged and then decanted through a filter, the filtrate being used for the thiochrome test.

15 cc of filtrate are placed in a centrifuge tube and washed with 15 cc isobutanol by bubbling with a stream of nitrogen for 2 or 3 minutes; after centrifuging the isobutanol layer is pipetted off and discarded. 5cc of the washed filtrate are then pipetted into each of two centrifuge tubes and the remaining volume of filtrate is transferred to a 25 cc measuring cylinder and the volume measured after the separation of any isobutanol. In this way it is possible to observe and allow for volume changes resulting from the washing of the filtrate with isobutanol.

To each of the two tubes is added 5 cc methyl alcohol and nitrogen is bubbled through whilst the following are added in the order indicated.

Tube 1 (test)	Tube 2 (blank)
1. 2.5cc 30% NaOH	2.5cc 30% NaOH
2. 1 cc 1% $K_3Fe(CN)_6$ *	Nil
3. 15 cc Isobutanol	15 cc Isobutanol.

* Freshly prepared every 48 hours.

After a reaction time of about 2 minutes, the tubes are centrifuged and the aqueous layers pipetted off and discarded. The isobutanol in each tube is then washed with 10 cc water, again bubbling with nitrogen; during this stage 3 drops of 3% H_2O_2 are added to tube 1 (test) but none to tube 2 (blank). After centrifuging the aqueous layer is removed and discarded and sufficient sodium sulphate is stirred into each tube to produce a clear solution. After a final centrifuging the isobutanol solution is decanted and the fluorescence measured in a Hilger photoelectric fluorimeter using a solution of quinine sulphate (25µg per ml) in N/10 sulphuric acid for comparison.

In the fluorimeter the light from the mercury discharge bulb is passed through Woods glass filters (H556) and one or two neutral filters (H508) are also used in front of the compensating photocell. The fluorescent light is transmitted through a

Wratten No.39 filter (Hilger H599).

A calibration curve is prepared in an identical manner, using standard aneurin hydrochloride solutions. For calibration purposes a solution of aneurin hydrochloride B.D.H. was prepared as described by Friedmann and Kmiecaik (14), dissolving 50 mg in 125 cc alcohol and diluting to 500 cc with sufficient hydrochloric acid to give a final acid concentration of N/100. Suitable dilutions were then made with N/10 hydrochloric acid. Typical results are shown in Fig. 30 .

Vitamin B_1 in urine

The general procedure in carrying out the thiochrome test with urine is identical with that employed for the filtrates from homogenised incubated tissue material, already described. 15 cc urine are washed with an equal volume of isobutanol then treated with methanol, sodium hydroxide and ferricyanide, the resulting thiochrome being extracted with isobutanol, washed, dried and measured.

Preliminary investigations were made to examine the variations in urinary vitamin B_1 excretion at different times of the day and to determine the best means of preserving samples of urine for the determination of vitamin B_1.

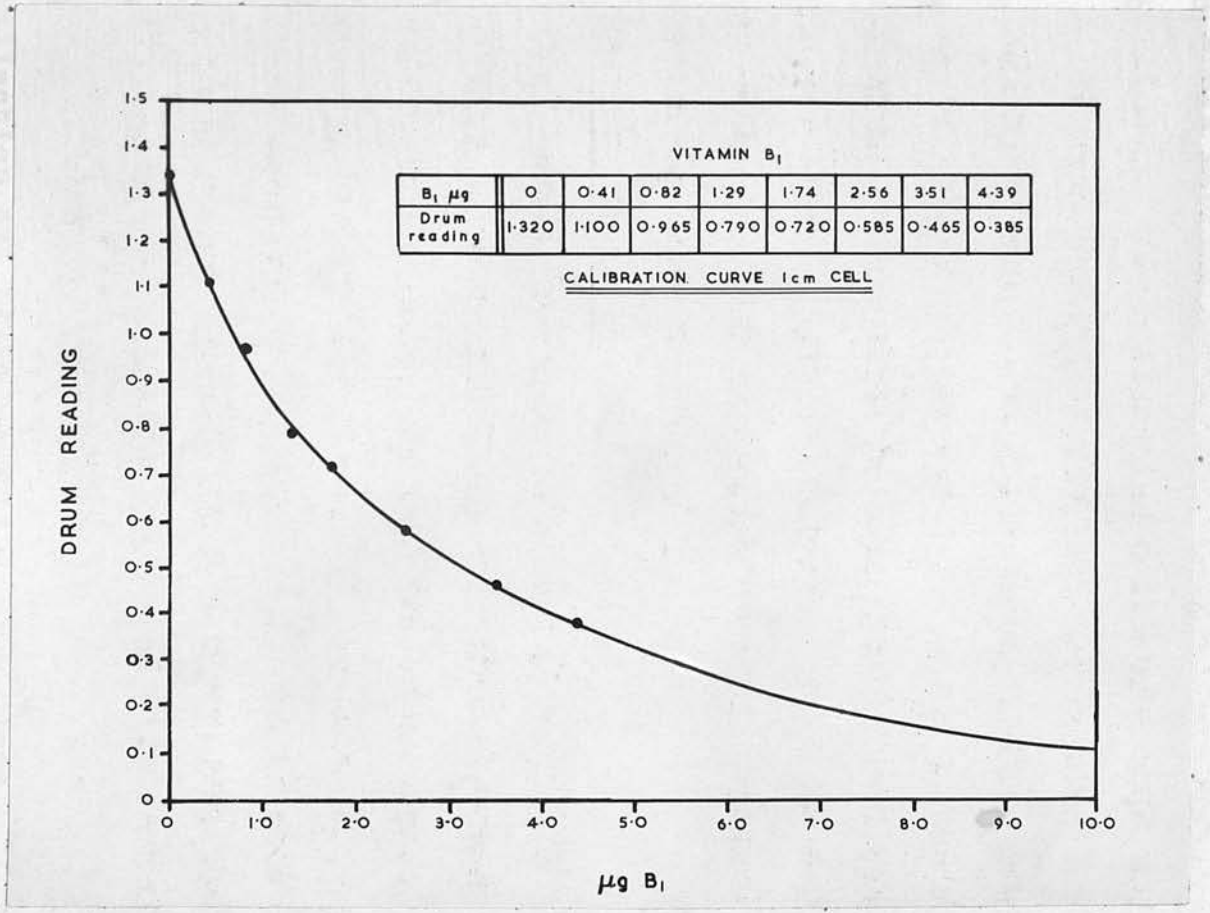

VITAMIN B1
B1 µg | 0 | 0·41 | 0·82 | 1·29 | 1·74 | 2·56 | 3·51 | 4·39
Drum reading | 1·320 | 1·100 | 0·965 | 0·790 | 0·720 | 0·585 | 0·465 | 0·385
CALIBRATION CURVE 1cm CELL
DRUM READING
µg B1
0 1·0 2·0 3·0 4·0 5·0 6·0 7·0 8·0 9·0 10·0
0 0·1 0·2 0·3 0·4 0·5 0·6 0·7 0·8 0·9 1·0 1·1 1·2 1·3 1·4 1·5

Fig. 30.

Daily variations in urine vitamin B_1 concentration.

Urine was collected from Sheep D (fed on hay) in three periods during the day and the vitamin B_1 content determined immediately after each collection. A second series of samples was taken at a later date and examined in the same way. The results given below, did not show any large variations.

Sample	Time of Collection	Vitamin B_1 ug/cc urine.
1	12-2 p.m.	0.692
2	2-7 p.m.	0.682
3	7p.m.-10a.m.	0.695
4	10a.m.-3p.m.	0.531
5	3 - 6 p.m.	0.677
6	6p.m.-10a.m.	0.547

So it was decided to make urine collections over 24 hours periods for the determination of vitamin B_1.

The acidification of urine to preserve the vitamin B_1 content

Since vitamin B_1 is easily destroyed in alkaline solutions and the urine of cattle and sheep is normally alkaline, acidification is obviously necessary in the collection and preservation of urine for vitamine B_1 determination. Tests were made in order to determine the degree of acidification necessary, and a suitable procedure for routine use. Urine collected from bullock No.5 fed on hay, was used for these tests. In the first test five separate fresh

excretions of urine collected at different times and subjected to varying degrees of acidification, were analysed immediately, after 1 hour and after 24 hours at room temperature with the results shown below.

Sample	Degree of acidification	vitamin B_1 (ug per 1cc) Initial value	after 1 hour	after 24 hours
1	None	1.91	1.57	1.50
2	None	1.46	1.01	1.07
3	to pH 6	1.60	1.37	1.47
4	to pH 4	2.29	2.28	2.32
5	pH 4	1.89	1.91	1.87

These figures show that without acidification a considerable loss of vitamin B_1 may occur particularly in the first hour after excretion. Some loss also occurs in urine acidified to pH 6 but not in urine acidified to pH 4.

In the second test, a single sample of fresh urine was divided into eight parts. One was analysed immediately, and the others were acidified to varying degrees and examined after 24 hours at room temperature. The figures obtained show clearly that the highest loss of B_1 occurred in the sample with no added acid, while there was little or no loss in the samples stored at pH4.0 and at pH $<$ 3.0 At pH values higher

than four there were significant losses.

No.	Time of storage at room temp.	pH	Vitamin B_1 µg per cc.
1	Nil	9.0	1.79
2	24 hours	9.0	1.10
3	"	7.5	1.50
4	"	7.0	1.56
5	"	6.0	1.52
6	"	5.0	1.60
7	"	4.0	1.74
8	"	<3.0	1.74

It was therefore decided to collect 24 hour urine samples with the addition of sufficient acid to produce a ~~sufficient~~ pH values of 4.0 or less. This could be achieved by adding to the urine bottle 3cc conc hydrochloric acid for every 100 cc urine expected.

B. Pyruvic acid

The main difficulty in the quantitative determination of pyruvic acid is the impossibility of separating it from other aldehyde or keto derivatives ~~in~~ (26); in colorimetric reactions these compounds form colours which are liable to affect the measurements. The most sensitive methods for determining pyruvic acid are based on the reaction with nitrophenylhydrazines. These methods are not specific for pyruvic acid but

they are highly specific for keto-acids as a group ~~Bokin et al~~ (8). All the nitrophenylhydrazones possess the property of forming deeply coloured water soluble salts in sodium hydroxide solution. A solution of any of the hydrazones in benzene or ether is therefore readily extracted with strong alkali; when however, extraction is made with 10% sodium carbonate solution, the hydrazones of glycocols and aldehydes are extracted only slightly, while the hydrazones of keto acids are extracted almost quantitatively.

The method used in this work was that described by Friedmann and Haugen (12) in 1943 for the determination of pyruvic acid in urine and blood samples.

Reagents

1. 2.4 dinitrophenyl hydrazine

This reagent reacts rapidly with keto-acids; its solution is stable. 100 mg are ground in a mortar with increasing small volumes of approximately 2N HCl until 100 cc have been added. The solution is filtered through a small filter paper and kept in a refrigerator when not in use.

2. Na_2CO_3 A 10% solution is prepared and stored in a Pyrex container (it is filtered if not clear immediately after preparation)

3. NaOH An approximately 1.5N solution is prepared and stored in a pyrex container.

Collection and treatment of samples

A. Blood 5 - 10 ml blood are drawn from the Jugular vein into a clean dry test tube and 3 ml transferred by pipette to a stoppered cylinder containing 15 ml cold 10% Trichloroacetic acid. After thorough mixing the contents of the cylinder are transferred to a tube and centrifuged for 10 minutes. The supernatant liquid is used for the determination of pyruvic acid and may, if necessary, be stored in a refrigerator for 2 days without demonstrable loss of pyruvic acid.

B. Urine 24 hour specimens are collected in receivers containing enough concentrated hydrochloric acid to acidify the urine. The use of a total of 3 cc conc. HCl for every 100 cc urine excretion anticipated was found to be satisfactory and the pyruvic acid content of the acidified urine did not change appreciably during another 24 hours storage in the refrigerator. For analysis 2 cc of acidified urine are added to 10cc of 10% trichloroacetic acid in a test tube containing 0.75 g. of adsorbent Fuller's earth; the mixture is well shaken and filtered immediately.

Method of analysis

3cc of the cold clear blood centrifugate or 3cc of the urine filtrate are transferred to a small Pyrex test tube (18 × 150 mm) and warmed for 10 minutes

in a water bath at approximately 25°C. 1 ml hydrazine reagent is then added and the mixture allowed to react for exactly 5 minutes in the water bath at 25°C. 3 ml benzene (A.R.) are then added and the contents of the tube thoroughly mixed by bubbling with a stream of nitrogen for 2 minutes. Most of the aqueous layer is removed with a capillary pipette and discarded; the test tube is then given a sudden circular motion to dislodge any drops of solution adhering to the walls, and the removal of the aqueous solution is then completed.

Exactly 6 ml 10% Na_2CO_3 are now added to the benzene solution and nitrogen rapidly bubbled for 2 minutes. After separation of the two phases a 5 cc pipette is passed quickly through the upper layer and cleared of any benzene solution by blowing a bubble or two of air through it before leaving to settle for 2 - 3 minutes. 5 cc of the sodium carbonate solution are then pipetted out and transferred to a small centrifuge tube. Exactly 5 cc 1.5N NaOH are added and the contents of the tube immediately mixed. The tube is centrifuged to avoid any error due to slight turbidity, and the colour measured 5 minutes later in a Hilger Spekker absorptiometer using the Hilger No.7 filter. In the comparison cell is used a reagent blank prepared in the same manner but

using 3 cc water instead of blood centrifugate or urine filtrate. A calibration curve is prepared using freshly distilled pyruvic acid in a series of dilutions in N/10 sulphuric acid.

The purity of the pyruvic acid used in this work was established by measurement of the bisulphite binding capacity using the method described by Clift and Cook (7). The standards prepared did not show evidence of deterioration on storage and gave the calibration curve shown in Fig. 31 . Since the method of analysis makes use of the distribution principle it is necessary to measure all solutions with care, and because of the volatility of the solvents and other sources of error, the determination should be carried to completion with a minimum of delay. Accurate timing is not so necessary in the analysis of blood but it is of the greatest importance in the analysis of urine or materials which contain dicarboxylic-keto-acids and other slowly reacting substances.

C. Urine alkalinity

Pipette 10 or 20 cc urine into a porcelain basin, and add an equal volume of water and 1cc of Brom-cresol-green indicator. Titrate with N/2 HCl to a greenish yellow end point, adding 0.5cc isobutanol to minimise frothing near the end point.

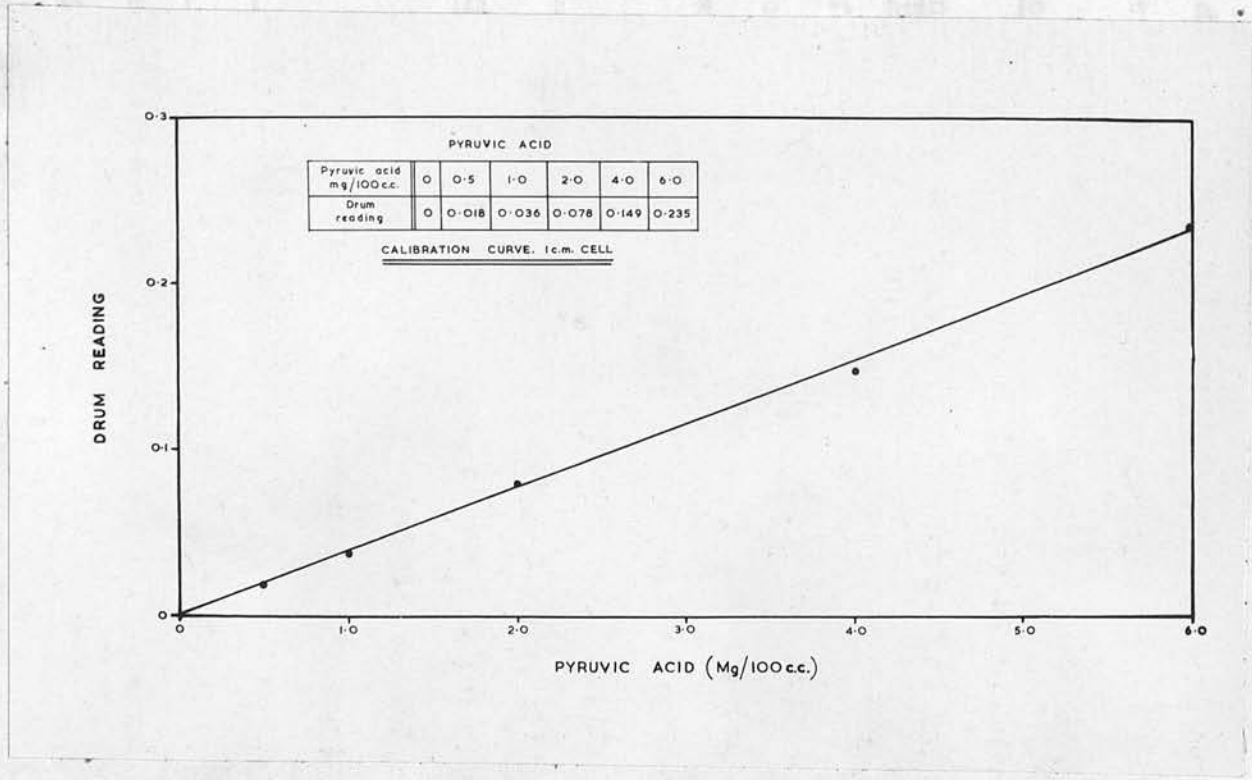

PYRUVIC ACID
Pyruvic acid mg/100 c.c. | 0 | 0·5 | 1·0 | 2·0 | 4·0 | 6·0
Drum reading | 0 | 0·018 | 0·036 | 0·078 | 0·149 | 0·235
CALIBRATION CURVE. 1 c.m. CELL
DRUM READING
0·3
0·2
0·1
0
1·0
2·0
3·0
4·0
5·0
6·0
PYRUVIC ACID (Mg/100 c.c.)

Fig. 31.

Results and discussion.

The study of the rôle of vitamin B_1 in relation to bracken poisoning necessitated some preliminary investigation of the factors affecting the vitamin B_1 excretion of normal animals.

A. Factors affecting the urinary excretion of vitamin B_1.

1. The individuality factor and the influence of vitamin B_1 administration.

The following figures for the vitamin B_1 concentrations in the 24 hours urine samples for 3 successive days show great differences between the four sheep although all were fed on hay under the same conditions.

Day	Urinary vitamin B_1 (μg/cc)			
	Sheep C	D	G	H
1	0.25	2.39	0.53	1.43
2	0.36	2.42	0.47	1.33
3	0.44	2.86	0.58	1.70
Average	0.35	2.55	0.53	1.49

These very large differences led to an examination of the effect of intravenous injections of vitamin B_1. Each sheep was injected with 10mg of the vitamin and 24 hours urine samples were collected for the following 5 days. Analysis of these urines gave the figures tabulated overleaf.

Day after injection	Vitamin B_1 µg/cc				Total urinary excretion of vitamin B_1(mg)			
	C	D	G	H	C	D	G	H
1st	10.67	9.41	4.49	8.07	4.38	6.21	4.35	6.06
2nd	2.27	2.93	0.87	2.19	1.13	1.84	0.65	1.66
3rd	0.74	3.44	0.70	3.00	0.45	2.37	0.67	2.31
4th	0.62	2.97	0.36	1.24	0.35	2.20	0.29	1.02
5th	0.41	2.82	0.42	1.82	0.27	1.93	0.28	1.20

The total daily excretion of vitamin B_1 in the urine was very high during the first day after injection and then decreased gradually until it reached the normal value for each individual. The concentration of vitamin B_1 in the urine showed similar changes, being very high on the 1st day after injection and showing a continuous decrease; the values on the fifth day after injection were very similar to the normal values found before injection, and given in the previous table.

These results show clearly that the large differences in urinary vitamin B_1 concentrations, found between the different animals on normal feeding are true individuality differences and that low values do not necessarily indicate vitamin B_1 deficiency, since injection of the vitamin does not produce any increased retention or any change in the urinary level after 3 or 4 days.

Wang and Yudkin (42) found similar variations in three humans in 1940 and related the differences of excretion to differences in the rate of absorption in the body or in the kidney threshold, or both.

The urinary excretion of injected B_1 vitamin, may be calculated if the average daily normal urinary B_1 excretion for each individual is deducted from the total daily excretion figures. In this way the

excretion above normal is reguarded as being derived from the injected vitamin. The following table shows that calculated in this manner, the average total excretion from an injection of 10 mg was 56.53% but there were appreciable differences between individuals.

Day after injection	Urinary excretion of injected vitamin B_1 (mg)			
	Sheep C	D	G	H
1st	4.23	4.52	3.84	4.94
2nd	0.96	0.23	0.26	0.54
3rd	0.24	0.61	0.17	1.17
4th	0.15	0.31	nil	nil
5th	0.04	0.18	nil	0.22
Total	5.62	5.85	4.27	6.87
% excretion	56.20	58.57	42.63	68.71
Average		56.53%		

In another experiment one sheep, C was fed on bracken and showed a deficiency of vitamin B_1 in the urine whereas another sheep H, was fed on hay and showed a normal urinary vitamin B_1 level. Both sheep were drenched with 10 mg vitamin B_1 given in the minimum quantity of water, but the vitamin B_1 concentration in the urine excreted did not show any change whatsoever. A few days later both sheep were fed with 10 mg vitamin B_1 absorbed in grass cubes and again there was no significant change in the urinary vitamin B_1. Since sheep H did not consume grass

cubes readily, on the following day this sheep was given a further 10 mg vitamin B_1 absorbed in hay but as the figures tabulated below indicate, there was no effect on the urinary excretion of the vitamin.

Day	Vitamin B_1 administered	Sheep C (bracken fed)		Sheep H (hay fed)	
		μgB_1/cc urine	Mg B_1 in total urine.	μgB_1/cc urine	MgB_1 in total urine
1	Nil	Nil	Nil	1.55	1.17
2	Nil	-	-	-	-
3	10mg as drench	-	-	1.59	1.25
4	Nil	Nil	Nil	1.99	1.47
5	Nil	-	-	-	-
6	Nil	-	-	-	-
7	Nil	Nil	Nil	0.94	0.68
8	Nil	-	-	-	-
9	10mg in grass cubes	Nil	Nil	-	-
10	10mg in hay (sheep H only)	Nil	Nil	1.65	1.11
11	Nil	Nil	Nil	1.57	1.11

Following these tests, both sheep were given dried yeast containing 14.58 µg vitamin B_1 per g. The dried yeast was homogenized with water and given as a drench after the failure of attempts to feed it as pellets, dried in the oven at 45°C. Sheep C was still fed on bracken, but during a period of 11 days was drenched with a total of 380g. dried yeast, containing 5.54 mg vitamin B_1. The urine still contained no vitamin B_1 throughout this period. Sheep H was drenched with 120 g dried yeast containing 1.75 mg vitamin B_1 in a single dose but the figures given below show no effect on the urinary vitamin B_1 excretion.

Day	Dried yeast fed	ug vitamin B_1/c.urine	mg vitamin B_1 in total urine
1	Nil	1.07	0.57
2	120	1.13	0.57
3	Nil	0.94	0.59

Thus oral administration of vitamin B_1, either as a drench or mixed with the feed does not affect the vitamin B_1 excreted in the urine of either bracken fed or hay fed sheep.

2. The effect of starvation

Since animals fed on bracken either fresh or dry are sometimes reluctant to eat sufficient quantities for maintenance and may therefore show effects due

to semi-starvation, this experiment was planned to study the effect of starvation on the vitamin B_1 excreted in the urine and also on the pyruvic acid excretion. Sheep H was starved for a period of six days, hay being fed both before and after this period. Urinary vitamin B_1 and pyruvic acid figures are tabulated below and also shown in fig. **32**.

Day	Food given	Vitamin B_1 in urine µg/cc	mg in total	Pyruvic acid in urine mg/100cc	mg in total
1	Hay	1.28	0.86	2.01	13.49
2	Nil	0.49	0.44	0.26	2.51
3	"	0.46	0.61	0.90	11.84
4	"	0.20	0.31	1.78	27.34
5	"	0.20	0.30	3.80	56.17
6	"	0.14	0.16	4.87	55.50
7	"	0.11	0.10	7.61	66.23
8	Hay	0.19	0.07	6.66	23.33
9	"	0.19	0.07	2.73	9.83
10	"	0.49	0.22	2.49	10.95
11	"	0.91	0.41	2.45	11.03

These results show a marked decrease in urinary vitamin B_1 during starvation and a corresponding rise in pyruvic acid. At the end of the starvation period the excretion of vitamin B_1 had fallen to about one tenth of its original value whilst pyruvic acid had increased to more than five times its original value.

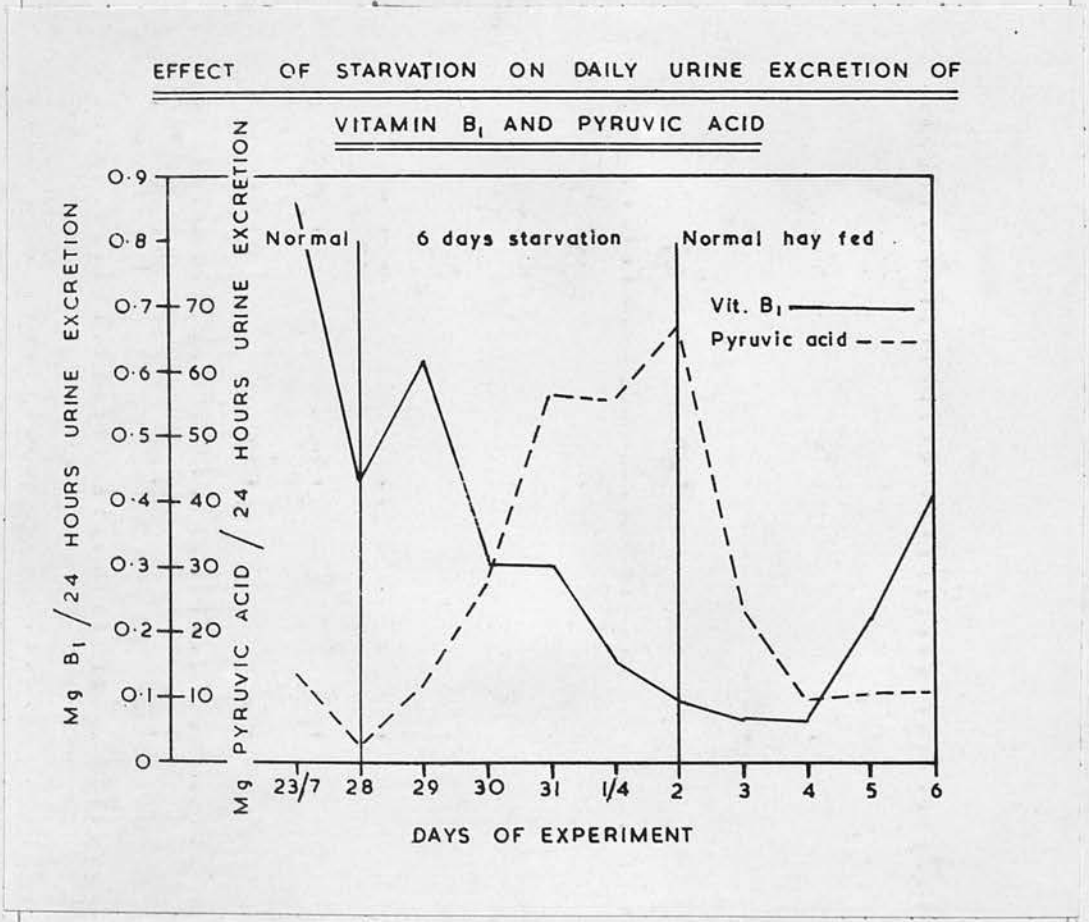


Fig. 32.

In the four days following the starvation period the pyruvic acid showed a sudden decline to normal values, although the recovery of vitamin B_1 excretion did not become apparent until the third day. The effect of starvation on the urinary excretion of vitamin B_1 and pyruvic acid may be explained on the assumption that growth of the rumen microflora responsible for the synthesis of vitamin B_1 is affected by starvation. As a result of the disturbance in vitamin B_1 synthesis carbohydrate metabolism is also affected, with a consequent increase in pyruvic acid excretion. The catabolism of body fat necessitated by starvation will, of course, lead to the production of ketone bodies, the influence from which may not be entirely eliminated in the method of analysis used to determine pyruvic acid.

3. The effect of urine alkalinity

Since vitamin B_1 is destroyed under alkaline conditions and the urine of animals fed on bracken is invariably alkaline, acidification is necessary in the preservation of urine samples and a suitable procedure has already been described. Even though urine samples are collected in acid, however, there remained the possibility of destruction of vitamin B_1 in the urine whilst it is still in the bladder, before excretion. Since only comparative values rather than

absolute values were required in this work, no attempt was made to collect urine from the bladder but an examination was made to see whether the feeding of bracken increased the alkalinity of urine above the level found when hay is fed. The effect of starvation on urine alkalinity was also examined. From the figures in appendix 8 (page 65) it will be seen that the urine alkalinity showed a sudden increase on the first day of feeding fresh or dried bracken. This sudden increase was however, only temporary, the alkalinity soon falling to normal. The average figures tabulated below show that the values during

M.Equivalent alkali excreted daily

Animal	Feed: Hay	Feed: Bracken 1st day	Feed: Bracken average
		Fresh bracken	
Bullock 1	2056	4153	3114
" 4	708	1559	1238
Sheep C	216	552	284
" E	385	617	198
" F	537	780	280
		Dried bracken	
Sheep D	156	172	54
" G	114	258	78.5
		NaCN	
Bullock 5	646	723	773
	Hay	starvation	
Sheep C	91	71	

bracken feeding are very similar to the normal figures obtained with ~~this~~ hay feeding. When bullock 5 was fed on hay and NaCN in grass cubes the milli-equivalent of alkali excreted (appendix 8) did not show any

sudden change, and all the figures were normal. The same happened when sheep C was starved for six days.

It is concluded that the variations in urine alkalinity during bracken feeding experiments will have no significance on the urinary vitamin B_1 figures.

B. The significance of vitamin B_1 in bracken poisoning in ruminants.

1. Fresh bracken

When bullock 4 and sheep E and F were fed on fresh bracken (2nd experiment) the urinary vitamin B_1 content (appendix 9 page 75) showed a gradual decrease to nil. In October the bullock was injected with a total of 425 mg vitamin B_1 whilst sheep E and F were each injected with 75 mg vitamin B_1. Although a considerable amount of the vitamin was excreted in the urines of these animals after injection, yet sheep E died two hours and sheep F one hour after injection, whilst the bullock died three days after the first injection. The figures for pyruvic acid excretion in the urine are given in appendix 9 (page 75) and summarised in the table overleaf.

In all these animals urinary pyruvic acid concentrations during bracken feeding were below normal, although blood levels showed a good deal of fluctuation. On the last day before death there were marked increases in both urinary and blood pyruvic acid in the cases of bullock 4 and sheep F, which died of bracken poisoning

Period	Pyruvic acid mg/100 ml					
	Urine			Blood		
	Bullock 4	Sheep E	Sheep F	Bullock 4	Sheep E	Sheep F.
Normal	9.2	7.2	5.9	1.7	- *	- *
Bracken feeding	2.3 - 5.9	3.9 - 7.0	2.4 - 5.5	0.90 - 3.2	0.95 - 2.99	1.1 - 2.6
Last day before death	29.5	5.3	10.6	8.5	1.5	4.2

* the average blood pyruvic acid for control sheep C fed on hay was 1.06 mg/100ml.

but not in the case of sheep E, which died from cause or causes other than bracken poisoning.

2. Dried bracken

Chopped dried bracken was fed continuously to sheep G and also to sheep C and D except for intervals when cubed bracken was fed. The vitamin B_1 values for the urines of these sheep (appendix 10 page 79) also shows a gradual decrease becoming nil after 9 to 16 days.

The urinary vitamin B_1 of sheep D and G remained nil until these animals died of bracken poisoning, although there were no symptoms of any sort associated with the absence of vitamin B_1 in the urine until a few days before death.

From the following table we find that the pyruvic acid level did not show any change during the bracken feeding except in the case of sheep G, where the pyruvic acid increased to 12.5mg/100ml in urine from the bladder at postmortem examination on the day of death.

Pyruvic acid mg/100ml urine

Period	Sheep C	Sheep D	Sheep G
Normal	2.438	2.438	2.438
Bracken feeding	0.769-3.129	0.842-10.83	1.482-7.046
day of death	-	1.665	12.517

In the case of sheep D although some relatively high pyruvic acid values were recorded these were

early in the bracken feeding period; towards the end of the bracken feeding of this sheep some very low puryvic acid values were found and even on the day of death there was not a high content of this metabolite. Sheep C did not develop bracken poisoning and the pyruvic acid in the urine of this animal was normal throughout the bracken feeding.

The blood pyruvic acid values for these three sheep, given in the table below, did not show any significant changes.

Sheep	Date	No. of days bracken fed	Blood pyruvic acid mg/100 ml.
C	3/4/50	Nil	1.39
	30/5/50	58	1.59
D			
died	3/4/50	19	1.85
9/7/50	30/5/50	76	1.46
G	3/4/50	19	1.56
died	24/5/50	-	-

3. NaCN feeding

This study was made with bullock 5 and sheep H to see if the feeding of cyanide had any effect on the vitamin B_1 and pyruvic acid levels in the urine. The results given in appendix II (page 85) are summarised in the following table and show that NaCN feeding has no effect on the vitamin B_1 and pyruvic acid values.

Animal	Feeding	Vit.B_1(µg/cc)	Pyruvic acid(mg/100cc
Bullock 5	Normal	2.114	9.2 *
	NaCN	1.697-2.632	2.178-4.648
Sheep H	Normal	1.487	1.935
	NaCN	0.945-1.511	1.702-2.379

* Normal value for bullock 4.

The blood pyruvic acid of bullock 5 was 0.622mg/100ml and for sheep H was 1.775; both these values are well within the normal range.

4. The vitamin B_1 content of postmortem tissue samples from experimental animals

In order to secure data for normal tissues of sheep and cattle with which to compare the values obtained for the tissues of bracken poisoned animals, control samples were obtained from the Edinburgh City slaughterhouse and analysed with the following results.

Vitamin B_1 in normal tissues (µg/g fresh material)

Animal		Muscle	Kidney	Liver	Heart
Bullock	1	1.97	5.82	3.65	6.41
	2	0.44	6.21	3.13	6.78
	3	0.88	4.69	2.55	5.19
	4	1.45	5.50	2.76	5.72
	5	0.88	3.87	2.55	4.32
	6	0.88	6.13	2.66	5.14
Mean		1.08	5.37	2.88	5.14
Sheep	1	1.65	5.53	2.69	5.38
	2	1.81	5.70	2.78	5.88
	3	2.62	7.37	4.59	5.56
	4	1.61	7.30	3.49	7.39
	5	2.82	6.67	2.94	5.42
	6	2.71	6.97	6.27	5.79

	Muscle	Kidney	Liver	Heart
Mean value for sheep	2.20	6.59	3.79	5.90

These figures are in agreement with those published by Michelsen (28) and Harris and Wang (16) and show the highest concentration of vitamin B_1 in kidney and heart, and least in the muscle as was also found by Leong. (23).

The vitamin B_1 contents of the postmortem tissues from experimental animals are shown in the following table.

Vitamin B_1 in post mortem tissues of bracken fed animals ug/g fresh material

Animal	Date of Death	Muscle	Kidney	Liver	Heart.
		Fresh bracken fed			
Bullock 4*	24/10/49	1.74	8.75	2.50	3.75
Sheep E*	21/10/49	0.20	-	1.40	-
Sheep F*	23/10/49	2.75	25.0	13.28	3.38
		Dried bracken fed			
Sheep G	24/5/50	Nil	2.56	Nil	1.44
Sheep D	9/7/50	0.96	1.97	1.71	2.95

* These animals were given intravenous injections of vitamin B_1 before death.

Bullock 4 and sheep F were injected intravenously with vitamin B_1 before death and the vitamin B_1 in the tissues was either normal or above normal except for the heart tissue. Sheep E was injected with vitamin B_1 only 2 hours before death and the tissue vitamin B_1 values were in this case lower than normal. Thus bullock 4 and sheep F died with bracken poisoning inspite of normal quantities of vitamin B_1 in the tissues whereas sheep E died showing no evidence of bracken poisoning although judged by tissue and urine analysis there was apparently a deficiency of vitamin B_1. It seems that although bracken feeding may have produced some degree of vitamin B_1 deficiency and certainly reduced urinary vitamin B_1 excretion, yet it is probably not responsible for producing the symptoms characteristic of bracken poisoning in ruminants.

Since injections of vitamin B_1 failed to cure Bullock 4 and sheep E and F, sheep G and D were not given any injection of vitamin B_1 when symptoms of bracken poisoning developed. The figures show that the tissues of these sheep had subnormal contents of vitamin B_1; the muscle and liver of sheep G contained no vitamin B_1 at all. In the following table the figures for sheep G and D are compared with the lowest values found in the normal sheep tissues examined.

Animal		vitamin B_1 µg/g fresh tissue muscle	kidney	liver	heart
Minimum normal value		1.61	5.53	2.69	5.38
Sheep G		Nil	2.56	Nil	1.44
Sheep D		0.96	1.97	1.71	2.95
Proportion of minimum normal value	G	-	1/2	-	1/4
	D	1/2	1/3	2/3	1/2

It will be noted that sheep D died with bracken poisoning in spite of the presence of 1/2 to 2/3 of normal tissue vitamin B_1 concentration. Westenbrink (43) working with rats and pigeons came to the conclusion that in five weeks of deficiency losses of vitamin B_1 from the tissues, lowered the concentration to 1/20 of the original value. In these experiments bracken feeding for 70 to 114 days produced reductions varying from 1/3 to complete loss.

Summary

1. Individuality has a marked effect on vitamin B_1 excretion in the urine.

2. Starvation reduces the vitamin B_1 and increases the pyruvic acid in urine.

3. Variations in urine alkalinity produced by bracken feeding have no significance in relation to the vitamin B_1 content of the urine.

4. The urinary excretion of vitamin B_1 by animals fed on fresh or dried bracken rapidly falls to a very low level but this may not indicate an absolute deficiency of vitamin B_1 since it is not associated with any symptoms of ill health until after a very prolonged period.

5. Pyruvic acid figures for urine and blood did not show any change during bracken feeding except on the day of death when the tissue metabolism would no doubt be affected in many ways.

6. Although bracken feeding appeared to produce some degree of vitamin B_1 deficiency in the experimental animals, yet this deficiency does not seem to be responsible for producing the symptoms characteristic of poisoning in ruminants.

7. NaCN feeding did not produce any significant change in the vitamin B_1 level of the urine and in the pyruvic acid concentration in blood and urine.

8. The vitamin B_1 in postmortem tissue samples from animals which had died with bracken poisoning varied from nil to 2/3 of normal.

9. Vitamin B_1 injections given to ruminants showing symptoms of bracken poisoning, did not produce any recovery.

10. This work indicates that the part played by vitamin B_1 in ruminant animals fed on bracken is quite different from that in rats and horses.

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Part IV

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PART V

The composition of the blood of bracken fed animals

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Introduction

It has been shown that bracken poisoning in ruminants is associated with widespread internal haemorrhages leading to rapid death. This fact shows clearly the need for a thorough investigation of the extent to which the disease may alter the composition of the blood.

The feeding of spoiled sweet clover hay or silage has caused deaths in cattle and sheep from haemorrhages due to an induced deficiency of Vitamin K. This produces a decrease in the prothrombin level of the blood and an increase in the coagulation time. The predominance of the haemorrhagic symptom in bracken poisoning in ruminants made it advisable to study the prothrombin level of the blood of bracken fed animals. In addition to this the specific gravities of blood and plasma were also examined and values for plasma protein, haemoglobin and haemotocrit were calculated.

Review of literature on blood prothrombin and the clotting mechanism

The clotting of blood when it is drawn from the vessels is an important property of the plasma. The chemical and physiochemical changes occurring during clotting are complex and there is still much

difference of opinion as to the exact nature of some of these processes. It is generally recognised that fibrin, the essential part of the clot, is formed from fibrinogen and that the conversion of fibrinogen to fibrin is brought about by the action of thrombin. Recent evidence as mentioned by Seegers (12) indicates that thrombin and its precursor prothrombin are carbohydrates containing proteins.

Thrombin is a plasma factor which is formed in the liver and vitamin K is necessary for its production though the way in which this vitamin acts in the formation of prothrombin is still uncertain.

According to the theory of Morawitz (7) thrombin is produced by the reaction of calcium with prothrombin in the presence of a substance called thrombokinase or thromboplastin, which is formed from injured body cells and disintegrated blood platelets. The clotting mechanism is therefore indicated as follows:-

1. Damaged blood platelets and tissue cells = thromboplastin.
2. Thromboplastin + prothrombin + Ca = thrombin.
3. thrombin + fibrinogen = fibrin.

This reaction converts a soluble protein, present in colloidal solution, into an insoluble protein (fibrin) appearing normally as a solid clot. The

changes are now universally believed to be enzymic in nature.

Howell (6) on the basis of his researches held that calcium ions alone are capable of converting prothrombin to thrombin but recent work by Ferguson (2, and 3) reaffirms the view of Morawitz that a thromboplastin factor, as well as ionized calcium, is necessary. According to Quick (10) prothrombin and calcium form a complex, which is acted on by thromboplastin.

It is also postuled that when blood is shed, a proenzyme present in it is activated and then catalyses the interaction of prothrombin, cephalin, and calcium to form free thrombin; this then reacts with fibrinogen forming the fibrin clot.

The time required for blood to coagulate varies widely between different species and there are appreciable variations in animals of the same species.

Methods of analysis

A. Prothrombin time

The methods used to determine the prothrombin level of blood are mostly based on the formation of a visible fibrin web. The prothrombin time is the exact time taken for the formation of the earliest detectable fibrin web after the addition of $CaCl_2$ to oxalated plasma in the presence of a source of

thromboplastin.

Campbell et al (1) determined the prothrombin level in rabbit plasma by the original procedure of Quick but using a series of dilutions (5% - 25%) in order to detect small changes. Fullerton (4) also used Quick's method for determining prothrombin. Witts and Hobson (14) concluded that if viper venom is used instead of brain extract as a source of thromboplastin it must be fortified by the addition of lecithin. They found that with venom alone the clotting time was longer than with venom and lecithin.

In the present work the modification of Quick's method (9) in which Russell viper venom is used as source of thromboplastin, was employed.

The detailed procedure is as follows. Since the venom does not keep well in solution, it is issued as a dry preparation, accompanied by a container of sterile distilled water (containing 0.5% phenol, as a preservative). The solution is prepared immediately before use by adding the solvent to the dried venom, producing a standard solution of concentration 1 : 10,000.

Two stages are recognised in this clotting process.

a. Prothrombin + calcium + thromboplastin = thrombin

b. Thrombin + fibrinogen = fibrin.

In the determination the amounts of Russell viper venom (and therefore of thromboplastin) and of calcium are kept constant; under these conditions the rate of clotting is related to the concentration of prothrombin.

1. 4.5 cc of blood are drawn into a dry syringe and mixed with 0.5 cc M/10 sodium oxalate

2. The oxalated blood is centrifuged at 1500 - 1700 R.P.M. for 5 minutes and the oxalated plasma drawn off. As small deficiencies are more easily detected in diluted plasma the prothrombin time is determined in the following series of plasma dilutions.

	Plasma%	Vol. of Plasma		Vol. of 0.9%NaCl
1	5%	0.05 cc	+	0.95 cc
2	10%	0.10 cc	+	0.90 cc
3	15%	0.15 cc	+	0.85 cc
4	20%	0.20 cc	+	0.80 cc
5	50%	0.50 cc	+	0.50 cc
6	100%	1.00 cc		-

3. 0.2cc plasma or its dilutions are pipetted off and transferred to a small test tube (75X10mm).

4. 0.2cc viper venom solution is added to the tube which is placed in a water bath at 37°C.

5. 0.2 cc M/40 $CaCl_2$ solution are added and a stop watch started, the tube being quickly shaken and returned to the water bath.

6. The tube is shaken and tilted in the water bath until distinct fibrin particles are seen. The exact time taken for the earliest detectable formation of a fibrin clot is the prothrombin time.

B. Specific gravities of whole blood and plasma for the determination of haemoglobin, plasma protein and haematocrit.

In the copper sulphate method of Phillips et al (8) drops of plasma or whole blood are allowed to fall into a graded series of solutions of copper sulphate of known specific gravity. Each drop on entering the solution becomes encased in a covering of copper-proteinate, and remains as a discrete drop without change of gravity for 15 to 20 seconds, during which time its rise or fall reveals its gravity relative to that of the solution. The size of the drop does not have to be constant, hence no special pipette is needed for delivering the drops. No temperature correction is needed, because the temperature coefficients of expansion of the copper sulphate solutions approximate to those of blood and plasma. This method is capable of measuring gravities to ± 0.00005, which is more than 10 times the accuracy required. The copper sulphate solution automatically cleans itself after each test, because within a minute or two after the test is completed

the material of the drop settles to the bottom as a precipitate. The standard $CuSO_4$ solutions are prepared by dilution of a stock solution which has at 25°C 1.1000 times the density of water.

Solutions:

1. Heller and Paul's Anti-coagulant (5)

3g. of ammonium oxalate + 2 g. potassium oxalate are dissolved in 250 cc water. 1.25 cc of this solution are used for every 25 cc of blood. The appropriate volume of oxalate mixture is placed in a centrifuge tube and dried in an incubator at 37°C.

2. Stock copper sulphate solution

159 g. of pure crystalline copper sulphate are dissolved in water and made to 1 litre at 25°C.

3. Copper sulphate dilutions

Since plasma has a specific gravity of 1.015 to 1.035 and whole blood falls in the range 1.035 to 1.075 the solutions should cover the specific gravity range 1.016 to 1.076. These dilutions are prepared as follows from the stock solution of copper sulphate.

Specific gravity	ml stock solution to make 100 cc
1.016	15 cc
1.020	19 cc
1.024	23 cc
1.028	27 cc
1.032	31 cc
1.036	35 cc
1.040	39 cc
1.044	43 cc

Specific gravity	ml stock solution to make 100 cc
1.048	47 cc
1.052	51 cc
1.056	55 cc
1.060	59 cc
1.064	63 cc
1.068	67 cc
1.072	71 cc
1.076	75 cc

The solutions are kept in well stoppered 120 cc bottles.

Procedure

Blood is freshly drawn into a centrifuge tube prepared with Heller and Paul's oxalate mixture and after determining the specific gravity of the whole blood, the remainder is centrifuged and the plasma removed for the determination of its specific gravity.

A small drop of whole blood or plasma is delivered from a height of about 1 C.M. above the solution. It breaks through the surface film of the solution and penetrates 2 - 3 cm below the surface then within 5 seconds it begins to rise or becomes stationary, or continues to fall. The gravity of the drop does not change appreciably for another 10 - 15 seconds and there is time to notice its behaviour during this interval. If the drop is lighter than the solution it will rise for this interval and then fall. If the drop is heavier it will continue to fall during the

interval and if the drop is of the same gravity as the standard solution it will remain stationary for this period and then fall.

Calculation of haemoglobin, plasma protein and haematocrit values

From the specific gravity figures of blood and plasma, hemoglobin and hematocrit values are obtained on the basis of an established relationship between these various quantities. Calculations are eliminated by the use of a special chart on which a line is drawn connecting the specific gravity values of plasma and whole blood. The hemoglobin and hematocrit values may then be read at the point of interception of the middle scale. Plasma protein content is directly proportional to the plasma specific gravity and is obtained directly from the scale on the chart, which is shown in Fig. 33 .

It should be noted that this chart was designed for measurements made on human blood and may not be strictly applicable to the specific gravities of the plasma and whole blood of cattle and sheep. Where comparative rather than absolute values are required, however, this limitation is probably of little consequence.

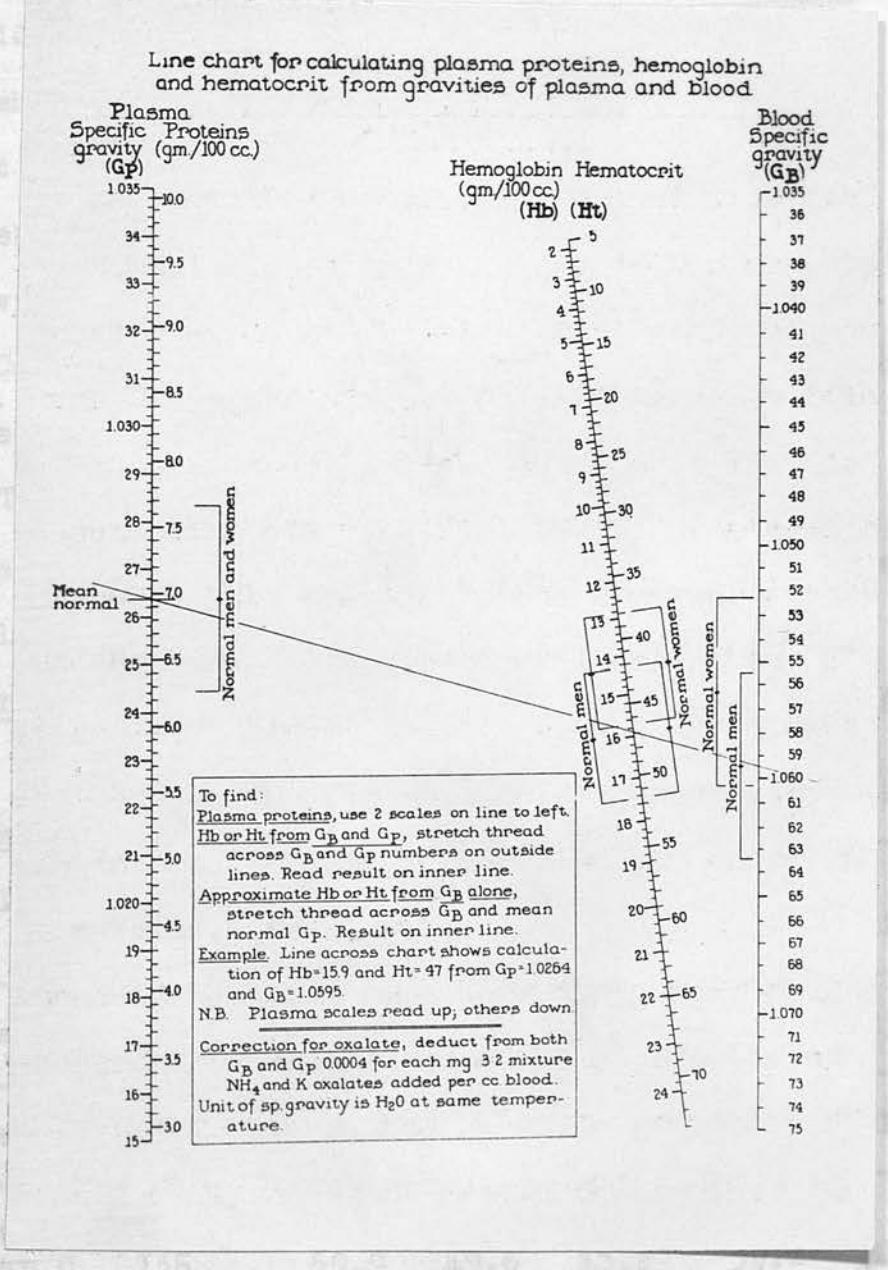
Line chart for calculating plasma proteins, hemoglobin and hematocrit from gravities of plasma and blood
Plasma Specific gravity (Gp)
Proteins (gm./100 cc.)
Hemoglobin (gm/100 cc.) (Hb)
Hematocrit (Ht)
Blood Specific gravity (GB)
Mean normal
Normal men and women
Normal men
Normal women
Normal women
Normal men
To find:
Plasma proteins, use 2 scales on line to left.
Hb or Ht from GB and Gp, stretch thread across GB and Gp numbers on outside lines. Read result on inner line.
Approximate Hb or Ht from GB alone, stretch thread across GB and mean normal Gp. Result on inner line.
Example. Line across chart shows calculation of Hb=15.9 and Ht=47 from Gp=1.0264 and GB=1.0595.
N.B. Plasma scales read up; others down.
Correction for oxalate, deduct from both GB and Gp 0.0004 for each mg 3.2 mixture NH4 and K oxalates added per cc blood.
Unit of sp. gravity is H2O at same temperature.

Fig. 33.

Results and discussion

Examinations were made of blood samples from bullock 4 and sheep E and F during the feeding of fresh bracken, in the 2nd experiment described in Part II. Samples were collected weekly for the determination of prothrombin time, and the specific gravities of whole blood and plasma.

Control samples were also collected at the same times from sheep C which was fed on hay.

The prothrombin times are tabulated fully in appendix 12, and the average values given in the following table.

Average clotting times of blood plasma in seconds.

Animal	Plasma concentration. 5%	10%	15%	20%	50%	100%
Bullock 4	52.1	37.0	30.8	28.7	23.1	22.6
Sheep E	70.6	47.1	39.2	33.4	24.9	23.3
Sheep F	98.3	48.5	36.8	29.3	22.8	22.0
Control Sheep C	135	59.9	49.6	45.2	39.2	31.4

Clotting times showed no significant changes during the whole period of this experiment until the animals developed haemorrhages and died. The values for sheep E and F are very similar and somewhat lower

than those for the control sheep C. The differences are greatest at the higher dilutions when the prothrombin time increases markedly as shown in Fig. 34. At all but the highest dilutions the prothrombin time of the bullock plasma was very similar to the values for sheep E and F.

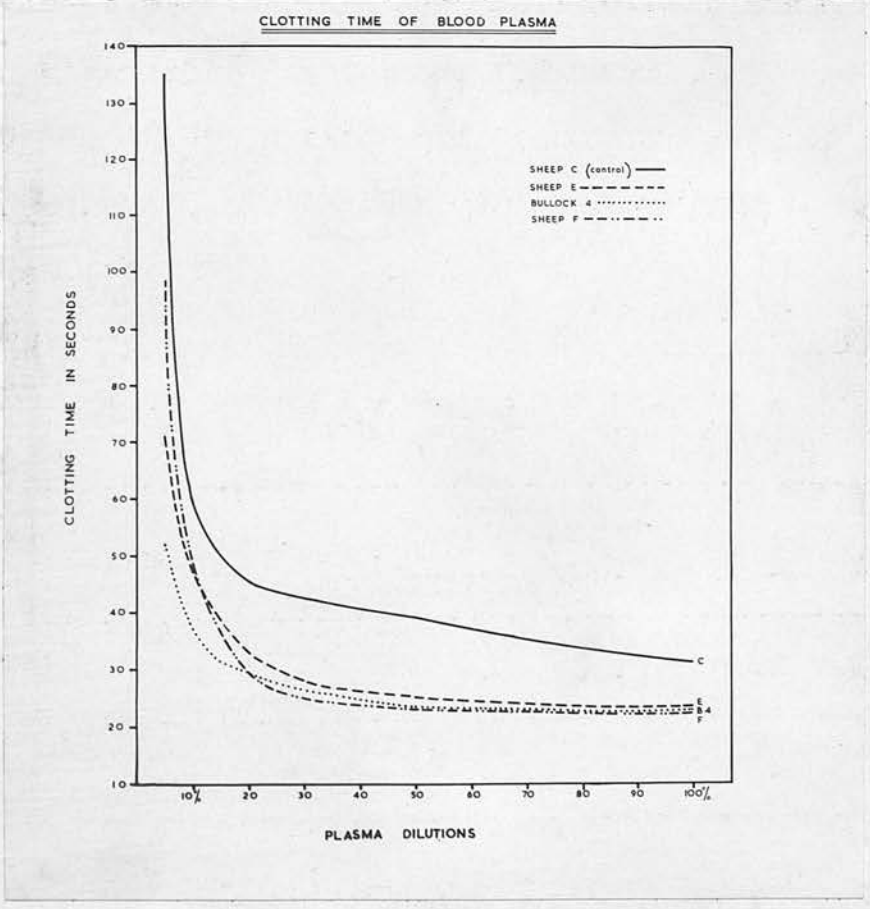

Fig. 34 - Average prothrombin times for plasma dilutions.

To indicate the changes which took place during the experimental bracken feeding the prothrombin times for the 10% and 15% plasma were averaged and are shown in the table below and in Fig.35.

Date	Bullock 4	Sheep E	Sheep F	Sheep C Control
6/9/49	31.5	34.6	23.6	57.6
13/9/49	36.8	38.8	44.8	50.1
20/9/49	24.3	-	-	-
27/9/49	30.2	41.1	49.3	56.1
4/10/49	39.1	56.3	37.8	-
14/10/49	40.7	48.8	36.9	-
21/10/49	29.2	44.3	63.4	-
24/10/49	39.5	Died	Died	-

Quick (9) showed that when the concentration of prothrombin is 60% of normal, the prothrombin time for whole plasma is only slightly prolonged but in diluted plasma the effect is much more marked. Figure 34 (page 223) also shows that changes in prothrombin time are more marked in the higher dilutions. The concentrations of 10% and 15% were considered to be the most suitable for showing up any changes so the means of these values were calculated.

These figures show that although there were some

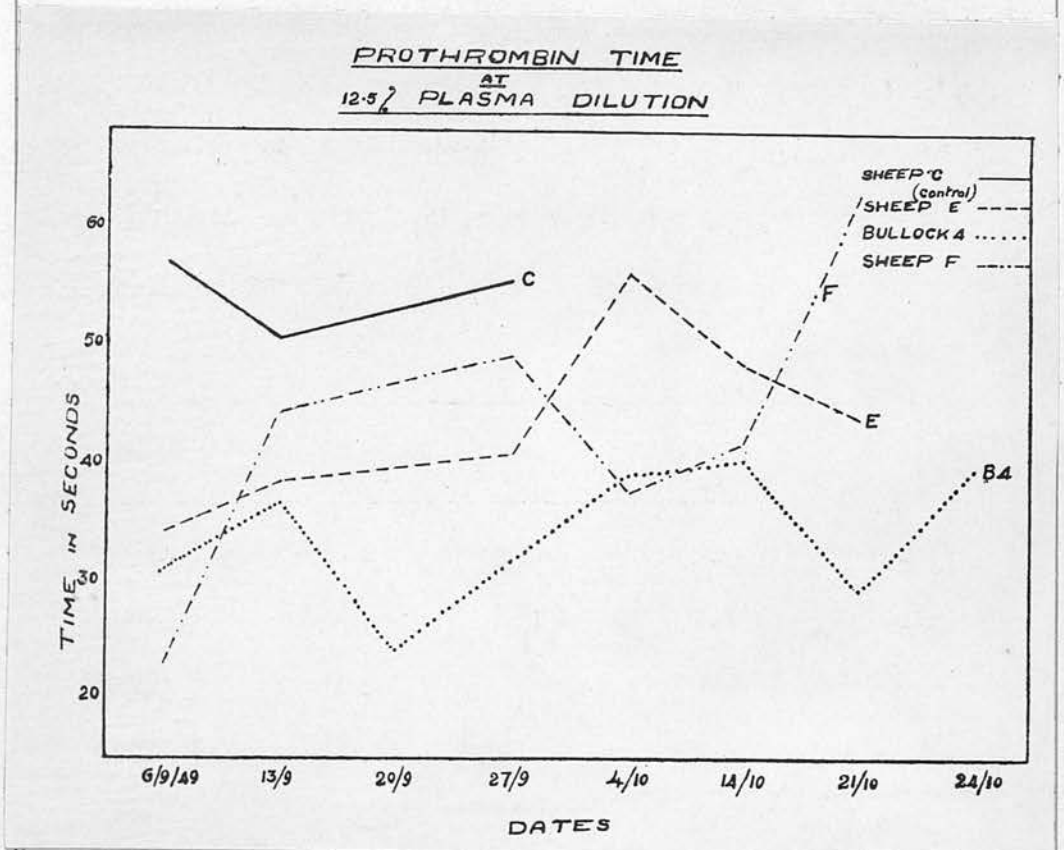

Fig. 35 - Prothrombin time variations during experimental period.

changes during the experiment they were relatively insignificant when compared with the values found for the normal control sheep C. Sheep F did, however show a sudden rise in the last sample although the final figure was not much greater than the average for sheep C.

In view of the absence of any significant changes prior to death from bracken poisoning these results rule out any possibility of a Vitamin K deficiency being involved in this disease. Values for specific gravity, plasmaprotein, haemoglobin and haematocrit

are given in appendix 13 , and the average figures are as follows:-

Animal	Period of sample	Specific gravity Blood	Specific gravity Plasma	Plasma Protein g/100cc	Hemoglobin g/100cc	Hematocrit
Bullock 4	Normal	1.052	1.027	7.18	12.05	35.09
	Exp. Period	1.053	1.028	7.46	12.51	37.1
	day of death	1.030	1.023	5.75	3.7	10.09
	Decrease %			22.90	70.4	72.7
Sheep E	Normal	1.047	1.024	6.10	10.80	31.9
	Exp. Period	1.047	1.0255	6.64	10.18	30.1
	day of death	1.048	1.024	6.10	11.20	33.0
Sheep F	Normal	1.045	1.022	5.49	10.50	30.9
	Exp. Period	1.046	1.024	5.92	10.46	30.85
	day of death	1.037	1.019	4.30	7.90	23.1
	Decrease %			27.40	24.5	24.8
Control Sheep C	Normal	1.045	1.023	5.66	10.24	30.2

The normal specific gravity values found for the bullock and sheep in this work are compared with those reported by other workers in the following table, which shows good agreement.

Specific gravity

Source of Figures	Bullock		Sheep	
	Blood	Plasma	Blood	Plasma
Present work	1.052	1.027	1.045	1.023
Turner and Herman (13)	-	1.0268 (cows)	-	-
Reichert and Brown (11)	1.061	-	1.043	-

The specific gravity and composition of the blood of sheep E remained normal throughout the experiment and even on the day of death. In the cases of bullock 4 and sheep F the values were also normal during the bracken feeding except on the day of death when there was a sudden fall in all values. In view of the extensive haemorrhages associated with death these late changes are not surprising.

The fall in haemoglobin and hematrocrit values were much greater in the case of bullock 4 than in sheep F, but the percentage decreases in plasma protein were very similar.

Summary

The blood of animals suffering from bracken poisoning does not show any significant change in prothrombin time so vitamin K deficiency cannot be involved in the production of this disease. Changes in specific gravity, plasma protein, haemoglobin, and hematocrit are restricted to the last stages of the disease when haemorrhages are extensive.

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PART VI

The urinary excretion of benzoic acid by bracken fed animals

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The urinary excretion of benzoic acid by Bracken fed animals

Introduction

In the analysis of urines for HCN and HCNS, previously described, the acidified urine after aeration was cooled and filtered. The heavy precipitate in the filters contained crystals which readily sublimed on heating. These cyrstals were identified as benzoic acid, a product to be expected from the hydrolysis of the normal hippuric acid of the urine. As the quantity of benzoic acid appeared to be unduly large, however, quantitative analyses were undertaken to determine both the total benzoic acid and the free benzoic acid in urine from animals fed on bracken.

Methods of analysis

A. Total benzoic acid

Different methods for the determination of total benzoic acid have been published. The titration method of Folin and Flanders (1), is quite accurate and simple but rather long and the gravimetric methods of Steen-back (5) and Hryntschak (2) though accurate, are far too tedious.

In the present work the titration method of Folin and Flanders as modified by Kingsbury and Swanson (3)

was used, the detailed procedure being as follows:-

1) 50 cc urine in a 500 cc Kjeldahl flask, to which 7.5 g NaOH and 0.5g magnesium oxide have been added, are boiled so as to reduce the volume to approx. 25cc in ½ hour. To stop bumping 2 or 3 pieces of glass are put in the flask.

2) Whilst still boiling 1cc 7% $KMnO_4$ is added, rinsing down any remaining on the neck with the minimum of water. After gentle agitation for a minute or two the flask is cooled under a tap and a test tube condenser placed in the neck.

3) 30 cc conc. HNO_3 are slowly added down the side of the condenser. The mixture rapidly clears up on the addition of the acid. After further boiling for 45 mins. with a good current of water through the condenser the flask is again cooled under the tap.

4) The condenser is rinsed down with 25cc of water to remove any benzoic acid sublimed on the bottom of the condenser, and the contents of the flask are transferred to a 500 cc separating funnel containing 25g of solid $(NH_4)_2SO_4$. The flask is rinsed with 20 cc water, which is also poured into a separating funnel.

5) After dissolving the $(NH_4)_2SO_4$ the benzoic acid is extracted successively with 50cc, 40cc, 30cc, 30cc and 30 cc of neutral well washed $CHCl_3$. The first two portions of $CHCl_3$ are used to rinse the Kjeldahl

flask before adding to the separating funnel.

6) The combined chloroform extracts in a second separating funnel are washed once with 150cc of the Folin-Flanders salt solution (1cc conc. HCl in 2 litres) saturated NaCl) then drawn off into a 200 cc graduated flask and made up to the mark.

7) An aliquot is titrated with sodium ethylate or alcoholic NaOH using phenolphthalein as indicator. A solution of pure benzoic acid in chloroform is used for standardisation.

B Free benzoic acid

The extraction of free benzoic acid from urine with $CHCl_3$ would be feasible if it were not for the troublesome emulsions formed. Raiziss and Dubin (4) have sought a solvent of benzoic acid which would form the least emulsion when shaken with fresh urine and found toluene most suitable. Toluene does not usually produce emulsions with fresh urine but may do so if there is contamination with faeces; a small amount of absolute alcohol will however usually break the emulsion.

The method followed in this work was that of Raiziss and Dubin (4) but the extract was made with ~~the~~ neutral $CHCl_3$, since toluene did not appear to possess any special advantage. The following procedure was followed.

100cc of fresh urine are pipetted into a short stemmed 500cc separating funnel and acidified with 1cc conc. HNO_3. Enough $(NH_4)_2SO_4$ (50-60g.) to saturate the

urine is added and free benzoic acid extracted with 5 portions of netural $CHCL_3$, 50cc, 50cc, 40cc, 30cc and 20cc respectively. The combined $CHCl_3$ extracts are then washed twice, using 100 cc each time, with saturated NaCl containing 0.5 cc conc. HCl per litre. The volume is made up to 200 cc in a measuring flask, and an aliquot titrated with N/10 sodium ethylate using phenolphthalein as indicator. The end point is a definite pink lasting 2 - 3 mins.

Results and discussion

The average figures for total benzoic acid in the urines of bracken fed animals are shown in the following table:-

Total benzoic acid in urine

Animal	Feed	g/100cc urine	g. in total daily urine.
Bullock 1	hay	1.41	73.89
	fresh bracken	1.65	84.01
Bullock 4	hay	1.92	26.76
	fresh bracken	1.99	28.38
Sheep E	hay	1.11	10.55
	fresh bracken	0.75	2.95
Sheep F	hay	0.91	11.74
	fresh bracken	1.47	11.08
Sheep C and D	hay	1.15	8.66

It is clear that neither the urinary concentration nor the total excretion of benzoic acid is significantly altered by feeding on fresh bracken. In the case of

sheep E the benzoic acid was considerably below normal when bracken was fed but this may be the result of the semi-starvation which occurred with this animal. The only other appreciable difference is the increased total excretion by bullock 1 when fed fresh bracken; this difference reflects the large increase in daily urine volume which took place when the diet was changed.

It seems that the total benzoic acid excreted by bracken fed animals is well within their capacity for detoxication. However, determinations of free benzoic acid were also made in case there should be abnormal quantities excreted in the free state as a result of some upset in the detoxication mechanism. Free benzoic acid was determined in the urines of animals fed on dried bracken as well as in the urine of bullock 5 when NaCN was fed. The results given below show that the excretion of free benzoic acid was not affected by feeding either on dried bracken or with cyanide. Thus the quantities of total and free benzoic acid in the urine of animals fed on fresh or dried bracken or cyanide are normal and cannot therefore be of any toxicological significance.

Animal	Feed	Free benzoic acid g/100cc urine	g. in total daily urine
Bullock 5	hay	0.134	3.551
	NaCN(+hay)	0.066	2.138
Sheep D	hay	0.113	0.859
	dried bracken	0.079	0.530
Sheep G	hay	0.074	0.585
	dried bracken	0.067	0.568

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General Summary

1. The mineral fraction of bracken has no toxic effect on ruminants.
2. The prothrombin time, the figures for plasma protein, haemoglobin, and hematocrit showed no significant change during bracken feeding, so vitamin K deficiency cannot be involved in the production of this disease.
3. Free and total benzoic acid in the urine of experimental animals during bracken feeding was normal and of no toxicological significance.
4. The vitamin B_1 figures for urine excreted by bracken fed animals suggest a rapid production of vitamin B_1 deficiency but the pyruvic acid figures for blood and urine did not show any significant change from normal, as would be expected if there were a true deficiency of vitamin B_1.
5. Injections of vitamin B_1 given to animals showing symptoms of bracken poisoning did not promote recovery.
6. Young bracken is rich in HCN and the values decrease gradually as the plant matures. This HCN found in young bracken may play some part in the production of bracken poisoning.
7. The poisoning of animals fed on fresh bracken is closely related to the stage of growth and hence to the HCN content of the plant. Animals which died

of bracken poisoning showed apparent positive balances of HCN equal to or greater than the lethal dose.

8. Bracken poisoning can be produced by feeding young artificially dried bracken containing a relatively low content of HCN, in large quantities during a long period of time.

9. It has been shown for the first time that bracken poisoning can be produced in sheep as well as cattle, with the same symptoms as in cattle.

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A P P E N D I X .

APPENDIX CONTENTS

APPENDIX 1

Live weight and dry matter consumed

Date	Live weight lb.	Date	Live weight lb.
	Sheep C - Fresh bracken fed		
	1st Experiment - 18/6/49 - 21/7/49		
18/6/49	91.0	8/7/49	63.0
19/6	87.5	9/7	62.25
20/6	84.0	10/7	62.25
21/6	81.0	11/7	62.0
22/6	78.0	12/7	61.75
23/6	78.0	13/7	61.5
24/6	76.5	14/7	60.25
25/6	74.0	15/7	59.0
26/6	72.75	16/7	58.5
27/6	72.5	17/7	57.75
28/6	72.0		
29/6	71.25	18/7	60.5
30/6	70.5	19/7	60.5
1/7	68.5	20/7	60.5
2/7	67.0	21/7	62.25
3/7	67.0		
4/7	66.75		
5/7	65.5		
6/7	64.5		
7/7	63.25		

hay fed

APPENDIX (Contd) 1

Date	Period of Experiment.	Live weight lb.	Dry matter Consumed g/day
		Sheep E - Fresh bracken fed 2nd Experiment - 18/8/49 - 21/10/49	
22/8/49	1	75.5	121.5
26/8	2	72.25	43.9
30/8	3	68.5	28.5
3/9	4	63.75	25.0
7/9	5	63.25	43.9
11/9	6	61.50	73.5
15/9	7	58.5	63.7
19/9	8	57.5	95.6
23/9	9	60.5	189.9
27/9	10	62.0	269.8
1/10	11	64.75	302.3
5/10	12	69.0	402.6
9/10	13	71.0	373.5
13/10	14	63.5	264.0
17/10	15	59.5	254.4
21/10	16	56.5	229.0
		Died on 21/10/49.	

APPENDIX 1 (Cont'd)

Date	Period of Experiment	Live weight lb.	Dry matter consumed g/day
	Sheep F - Fresh bracken fed 2nd Experiment - 18/8/49 - 23/10/49		
22/8/49	1	86.5	191.8
26/8	2	82. 5	87.2
30/8	3	80.0	147.6
3/9	4	80.0	262.4
7/9	5	81.0	352.8
11/9	6	86.0	459.1
15/9	7	91.0	438.2
19/9	8	93.0	665.6
23/9	9	95.5	806.5
27/9	10	94.0	740.0
1/10	11	92.0	689.6
5/10	12	92.25	729.0
9/10	13	93.0	644.0
13/10	14	89.5	516.0
17/10	15	84.75	480.0
20/10	16	86.0	633.0
21/10	17	88.0	274.0

Date	Period of Experiment.	Live weight lb.	Dry matter Consumed g Av. Per day
		Sheep G - Dried bracken fed 17/3/50 - 24/5/50	
17/3/50	1	108.75	603
20/3	2	99.50	39
24/3	3	94.5	63
28/3	4	93.25	213
2/4	5	91.75	410
5/4	6	94.0	512
9/4	7	94.75	555
13/4	8	95.50	593
18/4	9	96.75	613
22/4	10	97.0	746
26/4	~~11~~	99.0	945
30/4	12	100.0	964
4/5	13	101.0	879
8/5	14	100.5	1007
12/5	15	101.5	1035
16/5	16	102.0	984
20/5	17	101.0	806
24/5/50	18	96.0	79

Date	Period of Experiment.	Live weight lb.	Date	Period of Experiment.	Live weight lb.
		Sheep D - Dried bracken + Whole bracken cubes 16/3/50 - 9/7/50			
16/3/50	1	103.75	5/6/50	21	92.5
20/3	2	88.75	9/6	22	95.0
24/3	3	81.5	13/6	23	96.5
28/3	4	77.0	17/6	24	98.0
2/4	5	74.0	21/6	25	96.5
5/4	6	73.25	25/6	26	97.0
9/4	7	73.0	29/6	27	102.25
13/4	8	78.25	3/7	28	-
18/4	9	83.5	7/7	29	98.0
22/4	10	88.75	9/7/50	Died	-
26/4	11	85.0			
30/4	12	85.75			
4/5	13	84.25			
8/5	14	85.0			
12/5	15	89. 0			
16/5	16	89.0			
20/5	17	91.75			
24/5	18	96.50			
28/5	19	97.50			
1/6/50	20	91.0			

Date	Period of Experiment.	Live weight lb.	Date	Period of Experiment.	Live weight lb.
	Sheep C - Dried bracken + Extracted bracken cubes 3/4/50 - 31/7/50.				
5/4/50	1	83.75	25/6	21	100.5
9/4	2	81.5	29/6	22	104.75
13/4	3	78.5	3/7	23	-
18/4	4	78.25	7/7	24	-
22/4	5	84.0	11/7	25	105.0
26/4	6	88.5	15/7	26	104.0
30/4	7	91.5	19/7	27	-
4/5	8	91.0	23/7	28	104.0
8/5	9	91.0	26/7	29	106.0
12/5	10	89.0	30/7	30	108.5
16/5	11	91.0			
20/5	12	95.5			
24/5	13	96.5			
28/5	14	94.75			
1/6	15	99.0			
5/6	16	100.5			
9/6	17	107.5			
13/6	18	105.0			
17/6	19	100.0			
21/6	20	102.0			

APPENDIX 2

Fresh bracken - 1st Experiment

HCN Balance

Date		Fresh Bracken Consumed Kgm.	D.M. %	HCN in Bracken Consumed mg.	Daily Excretion of Urine cc.	HCN in Total Urine mg.	HCNS as HCN mg.	HCN Balance mg.
Feeding	Collection							
Bullock 1 - 18/6/49 - 18/7/49								
18/6 -	19/6/49	16.456	21.0	65.85	7120	1.11	28.5	+ 36.24
19/6 -	20/6	1.295	19.7	42.30	5170	0.33	31.1	+ 10.87
20/6 -	21/6	14.955	20.0	54.45	4800	0.45	46.7	+ 7.30
21/6 -	22/6	15.045	21.4	270.70	5600	0.70	129.7	+140.30
22/6 -	23/6	15.930	19.0	290.60	9100	0.28	127.6	+162.72
23/6 -	24/6	15.645	20.7	120.00	5850	nil	139.7	- 19.70
24/6 -	25/6	16.255	22.0	138.40	2830	nil	55.7	+ 82.70
25/6 -	26/6	14.845	23.5	231.00	4200	1.05	97.2	+132.75
26/6 -	27/6	14.885	25.5	170.70	3700	0.70	50.8	+119.20
27/6 -	28/6	15.485	26.0	97.70	7450	0.93	44.3	+ 52.47
28/6 -	29/6	17.250	20.0	81.55	6625	1.86	112.2	- 32.51
29/6 -	30/6	17.760	23.5	158.66	6004	0.94	68.7	+ 89.02
30/6 -	1/7	16.095	22.0	307.26	6625	1.66	111.0	+194.60
1/7 -	2/7	14.535	25.0	65.60	4050	0.26	86.3	- 20.96
2/7 -	3/7	15.315	23.9	154.65	4200	1.97	90.3	+ 62.38
3/7 -	4/7	14.065	23.5	245.30	5520	2.24	131.3	+111.76
4/7 -	5/7	15.715	22.5	76.80	5370	0.51	124.1	- 47.81
5/7 -	6/7	15.315	27.5	109.10	5290	0.83	122.5	- 14.23
6/7 -	7/7	15.135	25.4	129.10	4775	0.90	91.8	+ 36.40
7/7 -	8/7	15.115	27.0	102.60	5746	1.80	138.1	- 37.30

8/7 /

Date		Fresh Bracken Consumed Kgm.	D.M. %	HCN in Bracken Consumed mg.	Daily Excretion of Urine cc.	HCN in Total Urine mg.	HCNS as HCN in Total Urine mg.	HCN Balance mg.
eeding	Collection							
Bullock 1 - 18/6/49 - 18/7/49 (Cont'd)								
8/7 -	9/7/49	15.335	25.0	127.10	5810	1.63	39.9	+ 85.57
9/7 -	10/7	14.285	25.5	56.40	5350	2.17	50.4	+ 3.83
10/7 -	11/7	13.725	27.5	110.20	5350	3.18	61.2	+ 45.82
11/7 -	12/7	11.455	25.5	51.20	5140	3.21	65.4	- 17.41
12/7 -	13/7	10.295	28.0	51.70	4520	2.69	79.3	- 30.29
13/7 -	14/7	12.025	27.1	38.35	4245	2.65	17.1	+ 18.60
14/7 -	15/7	10.405	19.5	195.35	3870	0.60	49.6	+145.15
15/7 -	16/7	3.475	23.5	72.25	2666	0.42	23.2	+ 48.63
16/7 -	17/7	1.045	21.5	28.65	3250	0.31	10.3	+ 10.61
17/7 -	18/7	-	-	-	3950	0.31	9.40	- 9.71
Bullock died 18/7/49								
Totals				3643.52		35.69	2233.40	+1374.43

HCN Balance = + 1.374 gm

APPENDIX 2 (Cont'd)

Fresh bracken - 2nd Experiment

HCN Balance

Date		Fresh Bracken Consumed gm.	D.M. %	HCN in Bracken Consumed mg.	Daily Excretion of Urine cc.	HCN in Total Urine mg.	HCNS as HCN mg.	HCN Balance mg.
Feeding	Collection							
Bullock 4 - 18/8/49 - 22/10/49								
18/8 -	19/8/49	6646	29.5	70.0	2640			
19/8 -	20/8	2944	29.0	20.4	2000	1.4	58.2	+132.1
20/8 -	21/8	6615	27.5	56.9	2100			
21/8 -	22/8	3850	28.4	44.4	2180			
22/8 -	23/8	4450	27.5	34.8	2650			
23/8 -	24/8	4420	26.4	54.3	1290	0.5	91.3	+ 84.1
24/8 -	25/8	3760	29.0	31.3	1520			
25/8 -	26/8	4710	28.5	55.5	1850			
26/8 -	27/8	4851	28.0	39.1	1710			
27/8 -	28/8	4760	27.5	48.4	1690	1.0	92.1	+ 44.4
28/8 -	29/8	4300	28.0	22.9	1570			
29/8 -	30/8	3405	29.0	27.1	1520			
30/8 -	31/8	2800	28.5	29.1	1140			
31/8 -	1/9	3385	30.5	25.1	1200	1.2	56.0	+ 44.8
1/9 -	2/9	2970	28.0	11.4	1180			
2/9 -	3/9	3410	31.0	36.4	1240			
3/9 -	4/9	4595	28.0	30.2	1270			
4/9 -	5/9	4275	32.0	31.8	1560	0.8	102.3	- 16.6
5/9 -	6/9	4480	25.5	20.0	1330			
6/9 -	7/9	4625	31.5	4.5	1180			

7/9 /

Date		Fresh Bracken Consumed gm.	D.M. %	HCN in Bracken Consumed mg.	Daily Excretion of Urine cc.	HCN in Total Urine mg.	HCNS as HCN in Total Urine mg.	HCN Balance mg.
Feeding	Collection							
Bullock 4 - 18/8/49 - 22/10/49 (Cont'd)								
7/9 -	8/9/49	4745	34.5	27.0	1410			
8/9 -	9/9	4215	33.5	41.8	1350	0.8	52.6	+ 61.7
9/9 -	10/9	5030	26.5	31.2	1340			
10/9 -	11/9	5278	30.8	15.1	950			
11/9 -	12/9	5120	32.0	22.2	1300			
12/9 -	13/9	3819	26.3	1.0	1490	1.2	60.9	- 2.5
13/9 -	14/9	4325	31.0	12.9	1280			
14/9 -	15/9	5263	24.2	23.5	1360			
15/9 -	16/9	5760	27.0	5.0	1410			
16/9 -	17/9	5130	30.5	13.3	1500	0.9	67.2	- 39.1
17/9 -	18/9	5070	34.5	8.8	1590			
18/9 -	19/9	5095	33.7	1.9	1500			
19/9 -	20/9	5410	31.0	19.5	1504			
20/9 -	21/9	6190	29.5	7.7	1520	1.1	109.9	- 59.0
21/9 -	22/9	6080	27.0	15.1	1530			
22/9 -	23/9	5230	28.0	9.7	1230			
23/9 -	24/9	4990	30.2	13.6	1500			
24/9 -	25/9	5290	33.4	5.9	1130	0.6	93.3	- 72.1
25/9 -	26/9	5925	28.0	0.7	1460			
26/9 -	27/9	6415	30.0	1.6	1130			

Date		Fresh Bracken Consumed gm.	D.M. %	HCN in Bracken Consumed mg.	Daily Excretion of Urine cc.	HCN in Total Urine mg.	HCNS as HCN mg.	HCN Balance mg.
Feeding	Collection							
Bullock 4 - 18/8/49 - 22/10/49 (Cont'd)								
27/9 -	28/9/49	5935	31.0	0.4	1550			
28/9 -	29/9	5030	36.5	0.6	1480	0.7	55.8	- 54.9
29/9 -	30/9	4390	34.0	0.3	1135			
30/9 -	1/10	4520	37.0	0.3	1380			
1/10 -	2/10	4810	33.7	0.6	1400			
2/10 -	3/10	4690	34.5	1.8	1320	1.7	71.1	- 63.6
3/10 -	4/10	4420	37.5	5.0	1550			
4/10 -	5/10	4800	38.0	1.8	1300			
5/10 -	6/10	4652	37.0	0.6	1410			
6/10 -	7/10	3682	33.5	0.4	1210	1.7	48.5	- 48.1
7/10 -	8/10	2372	37.2	0.2	1100			
8/10 -	9/10	3342	36.5	0.9	960			
		New growth of bracken now fed						
9/10 -	10/10	5292	26.5	131.2	1030			
10/10 -	11/10	3857	25.0	97.6	1680	1.9	118.0	+299.4
11/10 -	12/10	4882	25.0	118.8	1420			
12/10 -	13/10	4312	27.0	71.7	1380			
13/10 -	14/10	5222	25.5	119.2	1850			
14/10 -	15/10	5132	28.0	91.7	1340	2.1	111.4	+292.5
15/10 -	16/10	5202	32.0	86.5	1720			
16/10 -	17/10	4977	27.5	108.6	1320			
17/10 /								

Date		Fresh Bracken Consumed gm.	D.M. %	HCN in Bracken Consumed mg.	Daily Excretion of Urine cc.	HCN in Total Urine mg.	HCNS as HCN mg.	HCN Balance mg.
Feeding	Collection							
				Bullock 4 - 18/8/49 - 22/10/49				
				New growth of bracken now fed (Cont'd)				
17/10 -	18/10/49	5322	27.5	110.8	1380)			
18/10 -	19/10	5697	26.0	148.3	1598)	1.9	135.3	+276.7
19/10 -	20/10	4347	33.5	75.5	1635)			
20/10 -	21/10	3732	32.5	79.3	1540)			
21/10 -	22/10	577	33.0	10.1	1460	0.2	33.1	- 23.2
22/10/49		Bullock offered hay - bracken feeding discontinued Died 24/10/49						
Totals				2233.3		19.7	1357.0	+856.6

HCN Balance = +0.857 gm.

Date		Fresh Bracken Consumed gm.	D.M. %	HCN in Bracken Consumed mg.	Daily Excretion of Urine cc.	HCN in Total Urine mg.	HCNS as HCN in Total Urine mg.	HCN Balance mg.
Feeding	Collection							
Sheep E - 18/8/49 - 21/10/49								
18/8 -	19/8/49	1000	29.5	1.054	1480			
19/8 -	20/8	109	29.0	0.756	1150	0.600	1.098	+ 6.785
20/8 -	21/8	297	27.5	2.465	660			
21/8 -	22/8	365	28.4	4.208	580			
22/8 -	23/8	245	27.5	1.913	580			
23/8 -	24/8	375	26.4	4.605	360	0.245	1.865	+ 7.848
24/8 -	25/8	166	29.0	1.379	1090			
25/8 -	26/8	175	28.5	2.061	600			
26/8 -	27/8	136	28.0	1.096	690			
27/8 -	28/8	145	27.5	1.475	280	0.348	-	+ 4.120
28/8 -	29/8	125	28.0	0.666	610			
29/8 -	30/8	155	29.0	1.231	290			
30/8 -	31/8	150	28.5	1.563	380			
31/8 -	1/9	135	30.5	1.004	475	0.220	0.252	+ 4.134
1/9 -	2/9	170	28.0	0.653	570			
2/9 -	3/9	130	31.0	1.386	350			
3/9 -	4/9	105	28.0	0.690	450			
4/9 -	5/9	220	32.0	1.637	370	0.139	0.211	+ 3.431
5/9 -	6/9	265	25.5	1.182	280			
6/9 -	7/9	275	31.5	0.274	390			

7/9 /

Date		Fresh Bracken Consumed gm	D.M. %	HCN in Bracken Consumed mg.	Daily Excretion of Urine cc.	HCN in Total Urine mg.	HCNS as HCN in Total Urine mg.	HCN Balance mg.
eeding	Collection							
				Sheep E - 18/8/49 - 21/10/49 (Cont'd)				
7/9 -	8/9/49	260	34.5	1.482	250			
8/9 -	9/9	265	33.5	2.629	180	0.025	0.115	+ 6.788
9/9 -	10/9	309	26.5	1.916	180			
10/9 -	11/9	316	30.8	0.901	200			
11/9 -	12/9	295	32.0	1.280	265			
12/9 -	13/9	299	26.3	0.075	220	0.059	0.676	+ 2.729
13/9 -	14/9	260	31.0	0.775	260			
14/9 -	15/9	299	24.2	1.334	208			
15/9 -	16/9	300	27.0	0.261	210			
16/9 -	17/9	350	30.5	0.910	180	0.043	1.183	+ 0.744
17/9 -	18/9	375	34.5	0.652	160			
18/9 -	19/9	396	33.7	0.147	145			
19/9 -	20/9	445	31.0	1.602	190			
20/9 -	21/9	650	29.5	0.806	180	0.095	3.605	+ 2.104
21/9 -	22/9	770	27.0	1.910	190			
22/9 -	23/9	799	28.0	1.486	210			
23/9 -	24/9	998	30.2	2.725	260			
24/9 -	25/9	928	33.4	1.039	290	0.171	4.993	- 1.062
25/9 -	26/9	1020	28.0	0.122	310			
26/9 -	27/9	865	30.0	0.216	240			
27/9 /								

Date		Fresh Bracken Consumed gm.	D.M. %	HCN in Bracken Consumed mg.	Daily Excretion of Urine cc.	HCN in Total Urine mg.	HCNS as HCN mg.	HCN Balance mg.
Feeding	Collection							
				Sheep E - 18/8/49 - 21/10/49 (Cont'd)				
27/9 -	28/9/49	946	31.0	0.057	190			
28/9 -	29/9	810	36.5	0.097	420	0.069	5.243	- 5.036
29/9 -	30/9	1060	34.0	0.064	280			
30/9 -	1/10	965	37.0	0.058	230			
1/10 -	2/10	860	33.7	0.103	350			
2/10 -	3/10	910	34.5	0.337	335	0.248	9.185	- 7.625
3/10 -	4/10	1004	37.5	1.124	340			
4/10 -	5/10	1200	38.0	0.244	310			
5/10 -	6/10	882	37.0	0.106	305			
6/10 -	7/10	868	33.5	0.105	260	0.120	7.411	- 6.929
7/10 -	8/10	1205	37.2	0.145	295			
8/10 -	9/10	985	36.5	0.246	430			
				New growth of bracken now fed				
9/10 -	10/10	950	26.5	23.560	395			
10/10 -	11/10	1030	25.0	26.059	570	0.380	7.826	+84.454
11/10 -	12/10	1155	25.0	24.925	1280			
12/10 -	13/10	1090	27.0	18.026	820			
13/10 -	14/10	900	25.5	20.538	900			
14/10 -	15/10	1005	28.0	17.949	430	-	8.188	+63.877
15/10 -	16/10	865	32.0	14.376	340			
16/10 -	17/10	880	27.5	19.202	550			
17/10 /								

Date		Fresh Bracken Consumed gm.	D.M. %	HCN in Bracken Consumed mg.	Daily Excretion of Urine cc.	HCN in Total Urine mg.	HCNS as HCN mg.	HCN Balance mg.
eeding	Collection							
			Sheep E - 18/8/49 - 21/10/49					
			New growth of bracken now fed (Cont'd)					
17/10 -	18/10/49	785	27.5	16.344	680)			
18/10 -	19/10	850	26.0	22.134	654.5)	0.072	10.527	+59.870
19/10 -	20/10	780	33.5	13.541	530)			
20/10 -	21/10	865	32.5	18.450	455)			
Totals				291.444		2.834	62.378	+226.232

HCN Balance = +0.226 gm.

Date		Fresh Bracken Consumed gm.	D.M. %	HCN in Bracken Consumed mg.	Daily Excretion of Urine cc.	HCN in Total Urine mg.	HCNS as HCN mg.	HCN Balance mg.
Feeding	Collection							
Sheep F - 18/8/49 - 23/10/49								
18/8 -	19/8/49	1000	29.5	10.540	2680			
19/8 -	20/8	1000	29.0	6.940	2600	1.884	12.933	+ 8.893
20/8 -	21/8	360	27.5	1.600	3250			
21/8 -	22/8	400	28.4	4.630	1600			
22/8 -	23/8	365	27.5	2.810	2000			
23/8 -	24/8	416	26.4	5.180	1300	1.565	5.860	+ 7.019
24/8 -	25/8	336	29.0	2.786	640			
25/8 -	26/8	315	28.5	3.668	650			
26/8 -	27/8	600	28.0	4.836	570			
27/8 -	28/8	450	27.5	4.619	710	0.286	7.373	+ 8.585
28/8 -	29/8	490	28.0	2.631	580			
29/8 -	30/8	520	29.0	4.158	450			
30/8 -	31/8	570	28.5	5.894	370			
31/8 -	1/9	845	30.5	6.240	310	0.303	10.868	+16.295
1/9 -	2/9	995	28.0	3.808	400			
2/9 -	3/9	1080	31.0	11.524	550			
3/9 -	4/9	1105	28.0	7.298	830			
4/9 -	5/9	1144	32.0	8.516	1030	0.645	21.273	+ 0.712
5/9 -	6/9	1259	25.5	5.636	720			
6/9 -	7/9	1215	31.5	1.180	390			

7/9 /

Date		Fresh Bracken Consumed gm.	D.M. %	HCN in Bracken Consumed mg.	Daily Excretion of Urine cc.	HCN in Total Urine mg.	HCNS as HCN in Total Urine mg.	HCN Balance mg.
Feeding	Collection							
				Sheep F - 18/8/49 - 23/10/49 (Cont'd)				
7/9 -	8/9/49	1479	34.5	8.400	330			
8/9 -	9/9	1244	33.5	12.240	450	0.441	11.363	+20.936
9/9 -	10/9	1255	26.5	7.800	540			
10/9 -	11/9	1504	30.8	4.300	460			
11/9 -	12/9	1645	32.0	7.180	565			
12/9 -	13/9	2005	26.3	0.500	750	0.646	16.616	+ 7.538
13/9 -	14/9	2250	31.0	6.740	620			
14/9 -	15/9	2330	24.2	10.380	668			
15/9 -	16/9	2290	27.0	2.010	750			
16/9 -	17/9	2290	30.5	6.000	340	0.805	11.550	- 0.015
17/9 -	18/9	2088	34.5	3.620	760			
18/9 -	19/9	1999	33.7	0.710	510			
19/9 -	20/9	2075	31.0	7.500	725			
20/9 -	21/9	2514	29.5	3.120	620	0.871	15.585	+ 7.384
21/9 -	22/9	3105	27.0	7.680	600			
22/9 -	23/9	2960	28.0	5.540	610			
23/9 -	24/9	2745	30.2	7.520	780			
24/9 -	25/9	1955	33.4	2.160	480	0.633	14.169	- 4.252
25/9 -	26/9	2386	28.0	0.290	430			
26/9 -	27/9	2330	30.0	0.580	580			
27/9 /								

Date		Fresh Bracken Consumed gm.	D.M. %	HCN in Bracken Consumed mg.	Daily Excretion of Urine cc.	HCN in Total Urine mg.	HCNS as HCN mg.	HCN Balance mg.
Feeding	Collection							
			Sheep F - 18/8/49 - 23/10/49 (Cont'd)					
27/9 -	28/9/49	2185	31.0	0.130	660			
28/9 -	29/9	1820	36.5	0.220	730	0.642	14.696	-14.778
29/9 -	30/9	1865	34.0	0.110	850			
30/9 -	31/9	1615	37.0	0.100	720			
1/10 -	2/10	1976	33.7	0.060	710			
2/10 -	3/10	1603	34.5	0.610	710	0.547	12.154	- 9.261
3/10 -	4/10	1864.5	37.5	2.060	600			
4/10 -	5/10	1867.5	38.0	0.710	500			
5/10 -	6/10	1610	37.0	0.160	510			
6/10 -	7/10	1590	33.5	0.160	505	0.576	8.495	- 8.041
7/10 -	8/10	1910	37.2	0.260	540			
8/10 -	9/10	1876	36.5	0.450	510			
			New growth of Bracken ~~fed~~ fed					
9/10 -	10/10	2020	26.5	50.100	860			
10/10 -	11/10	2460	25.0	62.250	2300	1.705	15.604	+155.421
11/10 -	12/10	2799	25.0	42.940	1300			
12/10 -	13/10	1050	27.0	17.440	1040			
13/10 -	14/10	1440	25.5	32.840	950			
14/10 -	15/10	1580	28.0	28.220	1050	1.445	19.233	+116.482
15/10 -	16/10	1530	32.0	25.440	1045			
16/10 -	17/10	2322	27.5	50.660	1192			
17/10 /								

Date		Fresh Bracken Consumed gm.	D.M. %	HCN in Bracken Consumed mg.	Daily Excretion of Urine cc.	HCN in Total Urine mg.	HCNS as HCN mg.	HCN Balance mg.
Feeding	Collection							
Sheep F - 18/8/49 - 23/10/49 (Cont'd)								
New growth of bracken fed								
17/10 -	18/10/49	2785	27.5	57.980	1225			
18/10 -	19/10	2980	26.0	77.560	1215	1.512	20.132	+175.936
19/10 -	20/10	1875	33.5	32.580	1025			
20/10 -	21/10	1380	32.5	29.460	970			
21/10 -	22/10	1570	33.0	27.620	1140	0.353	4.852	+ 37.455
22/10 -	23/10	1840	32.0	15.040	940			
23/10/49		Sheep died						
	Totals			763.924		14.859	222.756	+526.309

HCN Balance = + 0.526 gm.

APPENDIX 3

Dried bracken - Extracted and whole bracken cubes

Daily record sheets

Date Feeding	Collection	Dried Bracken Consumed gm.	Urine Daily Excretion cc.	Live Weight lb.	Temperature °F.
		Sheep C - Dried bracken			
3/4	4/4/50	275	610		
4/4	5/4	45	950		
5/4	6/4	48	670	83.75	
6/4	7/4	65	550		
7/4	8/4	93	1085		102.6
8/4	9/4	65	1290		
9/4	10/4	90	470	81.5	
10/4	11/4	153	520		
11/4	12/4	105	970		101.4
12/4	13/4	155	890		
13/4	14/4	183	860	78.5	
14/4	15/4	238	450		
15/4	16/4	340	820		
16/4	17/4	465	970		102.4
17/4	18/4	555	775		
18/4	19/4	505	640	78.25	
19/4	20/4	505	730		
20/4	21/4	470	1810		102.5
21/4	22/4	540	1390		
22/4	23/4	945	940	84.0	
23/4	/				

Date Feeding	Date Collection	Dried Bracken Consumed gm.	Urine Daily Excretion cc.	Live Weight lb.	Temperature °F.
		Sheep C - Dried bracken (Cont'd)			
23/4	24/4/50	840	780		
24/4	25/4	680	940		102.7
25/4	26/4	600	1200		
26/4	27/4	765	845	88.5	
27/4	28/4	818	995		
28/4	29/4	973	1100		102.5
29/4	30/4	785	740		
30/4	1/5	580	1210	91.5	
1/5	2/5	775	1100		
2/5	3/5	855	1200		102.8
3/5	4/5	785	1130		
4/5	5/5	840	1890	91.0	
5/5	6/5	873	1415		
6/5	7/5	1012	1260		102.6
7/5	8/5	863	1140		
8/5	9/5	828	1190	91.0	
9/5	10/5	935	1040		
10/5	11/5	875	1190		102.6
Total		20,522			

APPENDIX 3 (Cont'd)

Date		Food consumed		Urine Daily Excretion	Live Weight	Temperature
Feeding	Collection	+E.B.C. gm.	oD.B. gm.	cc.	lb.	°F
		Sheep C				
		Dried bracken + Extracted bracken cubes				
11/5 -	12/5/50	500	325	1720		102.6
12/5 -	13/5	1175	215	1690	89.0	
13/5 -	14/5	942	275	2160		
14/5 -	15/5	983	285	2220		102.5
15/5 -	16/5	1500	175	2080		
16/5 -	17/5	1445	140	2110	91.0	
17/5 -	18/5	2000	10	2320		
18/5 -	19/5	2000	45	2720		102.1
19/5 -	20/5	2000	nil	2635		
20/5 -	21/5	1840	nil	2630	95.5	
21/5 -	22/5	2000	5	2620		
22/5 -	23/5	2000	nil	2660		104.2
23/5 -	24/5	2000	nil	2790		103.5
24/5 -	25/5	2000	nil	2715	96.5	
25/5 -	26/5	2000	nil	2790		
26/5 -	27/5	2000	nil	3650		103.6
27/5 -	28/5	2000	70	3210		
28/5 -	29/5	2000	65	3500	94.75	
29/5 /						

+E.B.C. = Extracted bracken cubes

oD.B. = Dried bracken

APPENDIX 3 (Cont'd)

Date		Food consumed		Urine Daily Excretion	Live Weight	Temperature
Feeding	Collection	+E.B.C. gm.	°D.B. gm.	cc.	lb.	°F
		Sheep C				
		Dried bracken + Extracted bracken cubes (Cont'd)				
29/5 -	30/5/50	2000	55	3700		
30/5 -	31/5	2000	30	4000		104.6
31/5 -	1/6	2000	15	3890		
1/6 -	2/6	2000	75	3490	99.0	
2/6 -	3/6	2000	130	3690		
3/6 -	4/6	2000	160	4390		104.5
4/6 -	5/6	2000	155	4920		
5/6 -	6/6	2000	150	4510	100.5	
6/6 -	7/6	2000	165	3625		
7/6 -	8/6	2000	185	3190		104.5
8/6 -	9/6	2000	115	3310		
9/6 -	10/6	2000	115	4080	107.5	
10/6 -	11/6	2000	142	3380		
11/6 -	12/6	2000	150	3910		104.0
12/6 -	13/6	2000	145	3660		
13/6 -	14/6	2000	198	3595	105.0	
14/6 -	15/6	2000	210	3960		
15/6 -	16/6	2000	235	3720		103.7
16/6 /						

+E.B.C. = Extracted bracken cubes

°D.B. = Dried bracken

Date		Food consumed		Urine Daily Excretion	Live Weight	Temperature
Feeding	Collection	+E.B.C. gm.	°D.B. gm.	cc.	lb.	°F
		Sheep C				
		Dried bracken + Extracted bracken cubes (Cont'd)				
16/6 -	17/6/50	2500	185	3890		
17/6 -	18/6	2500	50	3920	100.0	
18/6 -	19/6	2440	40	3520		
19/6 -	20/6	2434	74	3690		103.6
20/6 -	21/6	2475	170	3550		
21/6 -	22/6	2480	100	3330	102.0	
22/6 -	23/6	2458	85	3620		
23/6 -	24/6	2260	90	3990		103.2
Total		85932	4834			

+E.B.C. = Extracted bracken cubes

°D.B. = Dried bracken

Date		Food consumed	Urine Daily	Live	
Feeding	Collection	Dried bracken gm.	Excretion cc.	Weight lbs	Temperature °F
		Sheep C.			
		Dried Bracken			
24/6/50	- 25/6/50	760	3140		
25/6	- 26/6	960	2520	100.5	
26/6	- 27/6	1040	1990		
27/6	- 28/6	1065	1900		103.1
28/6	- 29/6	1245	1635		
	Total	5070			

Sheep C.

Whole Bracken Cubes

Date		Food consumed	Urine Daily	Live	
Feeding	Collection	Whole bracken Cubes gm.	Excretion cc.	Weight lb.	Temperature °F
29/6/50	- 30/6/50	1980	1820	104.75	
30/6	- 1/7	2245	2270		
1/7	- 2/7	2238	2645		103.6
2/7	- 3/7	2248	2710		
3/7	- 4/7	2475	2800	———	
4/7	- 5/7	2470	3240		
5/7	- 6/7	1940	2780		
6/7	- 7/7	590	1960		
7/7	- 8/7	507	1493	———	
8/7	- 9/7	578	1800		103.5
	TOTAL	17,271			

Date Feeding	Date Collection	Food Consumed Dried Bracken gm.	Urine Daily Excretion cc.	Live Weight lb.	Temperature °F
		Sheep C.			
		Dried Bracken			
9/7/50	- 10/7/50	1030	2340		
10/7	- 11/7	1176	1660		
11/7	- 12/7	1175	1760	105.0	
12/7	- 13/7	970	1900		
13/7	- 14/7	555	2340		102.8
14/7	- 15/7	705	1450		
15/7	- 16/7	760	2135	104.0	
16/7	- 17/7	715	2020		
17/7	- 18/7	605	1500		102.8
18/7	- 19/7	300	1270		
19/7	- 20/7	550	2020	———	
20/7	- 21/7	815	1975		
21/7	- 22/7	1135	1525		
22/7	- 23/7	1078	1575		103.2
23/7	- 24/7	1418	1475	104.0	
24/7	- 25/7	1295	1640		
25/7	- 26/7	1338	1565		
26/7	- 27/7	1360	1910	106.0	
27/7	- 28/7	1575	1715		103.4
28/7	- 29/7	1678	1800		
29/7	- 30/7	1623	1765		102.8
30/7	- 31/7	1600	1670	108.5	102.8
31/7	- 1/8	1480	1750		103.1
	TOTAL Consumed in 23 days.	24,936 gm. D.B.			

APPENDIX 3 (Cont'd)

Date Feeding	Date Collection	Dried Bracken Consumed gm.	Urine Daily Excretion cc.	Live Weight lb.	Temperature °F
		Sheep D - Dried bracken			
16/3	17/3/50	270	915	103.75	
17/3	18/3	70	1200		
18/3	19/3	45	1070		
19/3	20/3	28	600		
20/3	21/3	48	520	88.75	
21/3	22/3	28	480		
22/3	23/3	45	380		
23/3	24/3	41	320		
24/3	25/3	52	290	81.5	
25/3	26/3	35	310		
26/3	27/3	43	530		
27/3	28/3	51	350		
28/3	29/3	60	250	77.0	
29/3	30/3	70	290		
30/3	31/3	75	250		
31/3	1/4	65	220		
1/4	2/4	98	210		
2/4	3/4	90	270	74.0	
3/4	4/4	123	240		102.8
4/4	5/4	115	345		
5/4	/				

APPENDIX 3 (Cont'd)

Date Feeding	Date Collection	Dried Bracken Consumed gm.	Urine Daily Excretion cc.	Live Weight lb.	Temperature °F
		Sheep D - Dried bracken (Cont'd)			
5/4	6/4/50	118	270	73.25	
6/4	7/4	138	265		
7/4	8/4	140	330		103.2
8/4	9/4	155	290		
9/4	10/4	255	340	73.0	
10/4	11/4	365	340		
11/4	12/4	453	380		102.7
12/4	13/4	645	520		
13/4	14/4	698	650	78.25	
14/4	15/4	625	640		
15/4	16/4	675	630		
16/4	17/4	670	630		103.0
17/4	18/4	740	690		
18/4	19/4	785	700	83.5	
19/4	20/4	755	740		
20/4	21/4	1025	770		103.2
21/4	22/4	1015	875		
22/4	23/4	1000	840	88.75	
23/4	24/4	240	870		
24/4	25/4	900	820		102.4
25/4	/				

APPENDIX 3 (Cont'd)

Date Feeding	Date Collection	Dried Bracken Consumed gm.	Urine Daily Excretion cc.	Live Weight lb.	Temperature °F
		Sheep D - Dried bracken (Cont'd)			
25/4	26/4/50	870	880		
26/4	27/4	780	890	85.0	
27/4	28/4	896	935		
28/4	29/4	795	930		103.0
29/4	30/4	875	880		
30/4	1/5	655	850	85.75	
1/5	2/5	600	910		
2/5	3/5	675	910		103.5
3/5	4/5	850	910		
4/5	5/5	985	780	84.25	
5/5	6/5	973	860		
6/5	7/5	761	938		103.2
7/5	8/5	595	835		
8/5	9/5	705	810	85.0	
9/5	10/5	925	850		
10/5	11/5	1015	870		102.1
Total		25,804			

APPENDIX 3 (Cont'd)

Date	Food consumed		Urine Daily Excretion	Live Weight	Temperature
Feeding Collection	+W.B.C. gm.	°D.B. gm.	cc.	lb.	°F
		Sheep D (Cont'd)			
		Dried bracken + Whole bracken cubes			
11/5 - 12/5/50	500	410	1090		
12/5 - 13/5	1375	105	1440	89.0	
13/5 - 14/5	710	320	1790		
14/5 - 15/5	890	340	1415		103.0
15/5 - 16/5	765	210	1410		
16/5 - 17/5	975	200	1460	89.0	
17/5 - 18/5	961	220	1500		
18/5 - 19/5	1250	235	1560		102.0
19/5 - 20/5	1180	225	1550		
20/5 - 21/5	1115	240	1450	91.75	
21/5 - 22/5	1430	230	1510		
22/5 - 23/5	1150	205	1345		102.4
23/5 - 24/5	1265	210	1350		
24/5 - 25/5	1223	250	1360	96.50	
25/5 - 26/5	1500	230	1535		
26/5 - 27/5	1500	190	1730		103.0
27/5 - 28/5	1500	185	1680		
28/5 - 29/5	895	225	1480	97.50	
29/5 /					

+W.B.C. = Whole bracken cubes

°D.B. = Dried bracken

APPENDIX 3 (Cont'd)

Date	Food consumed		Urine Daily Excretion	Live Weight	Temperature
Feeding Collection	+W.B.C. gm.	°D.B. gm.	cc.	lb.	°F
Sheep D					
Dried bracken + Whole bracken cubes (Cont'd)					
29/5 - 30/5/50	305	250	1070		
30/5 - 31/5	370	230	865		104.0
31/5 - 1/6	650	235	1010		
1/6 - 2/6	1000	250	1640	91.0	
2/6 - 3/6	1500	225	2070		104.4
3/6 - 4/6	1500	190	2280		103.8
4/6 - 5/6	1500	175	2100		
5/6 - 6/6	1500	215	2100	92.5	
6/6 - 7/6	1500	175	2050		
7/6 - 8/6	1500	200	2120		103.7
8/6 - 9/6	1500	230	2130		
9/6 - 10/6	1500	230	2050	95.0	
10/6 - 11/6	1500	227	2020		
11/6 - 12/6	1500	215	1980		104.2
12/6 - 13/6	1500	210	2200		
13/6 - 14/6	1500	230	1930	96.5	
14/6 - 15/6	1500	210	1890		
15/6 - 16/6	2000	190	2110		104.1
16/6 /					

+W.B.C. = Whole bracken cubes

°D.B. = Dried bracken

APPENDIX 3 (Cont'd)

Date		Food consumed		Urine Daily Excretion	Live Weight	Temperature
Feeding	Collection	+W.B.C. gm.	°D.B. gm.	cc.	lb.	°F
		Sheep D				
		Dried bracken + Whole bracken cubes (Cont'd)				
16/6 -	17/6/50	2000	155	2140		
17/6 -	18/6	2391	25	2135	98.0	
18/6 -	19/6	1950	85	2160		
19/6 -	20/6	1773	100	2212		104.4
20/6 -	21/6	1850	155	2270		
21/6 -	22/6	2225	170	2130	96.5	
22/6 -	23/6	2400	140	2390		
23/6 -	24/6	2185	110	2420		104.2
Total		60,783	9,057			

+W.B.C. = Whole bracken cubes

°D.B. = Dried bracken

APPENDIX 3 (Cont'd)

Date Feeding	Date Collection	Dried Bracken Consumed gm.	Urine Daily Excretion cc.	Live Weight lb.	Temperature °F
		Sheep D - Dried bracken (Cont'd)			
24/6	25/6/50	860	1760		
25/6	26/6	1175	1400	97.0	
26/6	27/6	1225	1370		
27/6	28/6	1515	1240		103.0
28/6	29/6	1540	1485		
29/6	30/6	1215	1260	102.25	
30/6	1/7	1335	1240		
1/7	2/7	1263	1240		103.6
2/7	3/7	1448	1320		
3/7	4/7	1216	1255		
4/7	5/7	1210	1250		
5/7	6/7	968	1240		103.7
6/7	7/7	1013	1180		
7/7	8/7	973	1405	98.0	
8/7	9/7	141	1060		
9/7	10/7	Sheep died on 9/7/50			109.0
Total		17,097			

APPENDIX 3 (Cont'd)

Date Feeding	Date Collection	Dried Bracken Consumed gm.	Urine Daily Excretion cc.	Live Weight lb.	Temperature °F
		Sheep G - Dried bracken			
16/3	17/3/50	603	1500	110.75	
17/3	18/3	105	1850	108.75	
18/3	19/3	20	1060		
19/3	20/3	10	920		
20/3	21/3	87	810	99.5	
21/3	22/3	48	720		
22/3	23/3	55	860		
23/3	24/3	63	530		
24/3	25/3	95	800	94.5	
25/3	26/3	130	660		
26/3	27/3	228	530		
27/3	28/3	400	650		
28/3	29/3	480	780	93.25	
29/3	30/3	555	1270		
30/3	31/3	275	920		
31/3	1/4	270	650		
1/4	2/4	468	570		
2/4	3/4	461	600	91.75	
3/4	4/4	508	600		103.4
4/4	5/4	568	730		
5/4	/				

APPENDIX 3 (Cont'd)

Date Feeding	Date Collection	Dried Bracken Consumed gm.	Urine Daily Excretion cc.	Live Weight lb.	Temperature °F
		Sheep G - Dried bracken (Cont'd)			
5/4	6/4/50	525	900	94.0	
6/4	7/4	576	1020		
7/4	8/4	553	1090		103.6
8/4	9/4	565	960		
9/4	10/4	590	980	94.75	
10/4	11/4	600	1050		
11/4	12/4	585	1060		103.1
12/4	13/4	596	950		
13/4	14/4	530	960	95.50	
14/4	15/4	588	910		
15/4	16/4	585	900		
16/4	17/4	600	960		103.0
17/4	18/4	763	1085		
18/4	19/4	735	1290	96.75	
19/4	20/4	725	1270		
20/4	21/4	710	1190		103.0
21/4	22/4	815	1490		
22/4	23/4	1045	1280	97.0	
23/4	24/4	860	1420		
24/4	25/4	915	1390		102.8
25/4	/				

APPENDIX 3 (Cont'd)

Date		Dried Bracken Consumed gm.	Urine Daily Excretion cc.	Live Weight lb.	Temperature °F
Feeding	Collection				
		Sheep G - Dried bracken (Cont'd)			
25/4 -	26/4	960	1440		
26/4 -	27/4	935	1400	99.0	
27/4 -	28/4	1005	1420		
28/4 -	29/4	960	1565		103.0
29/4 -	30/4	955	1430		
30/4 -	1/5	730	1385	100.0	
1/5 -	2/5	750	1440		
2/5 -	3/5	1030	1400		103.4
3/5 -	4/5	1005	1555		
4/5 -	5/5	1050	1395	101.0	
5/5 -	6/5	1023	1595		
6/5 -	7/5	998	1615		103.4
7/5 -	8/5	955	1360		
8/5 -	9/5	1050	1405	100.5	
9/5 -	10/5	1020	1500		
10/5 -	11/5	1035	1315		102.7
11/5 -	12/5	1035	1400		
12/5 -	13/5	975	1320	101.5	
13/5 -	14/5	945	1400		
14/5 -	15/5	965	1510		102.9
15/5 -	16/5	1050	1530		
16/5 -	17/5	1025	1540	102.0	
17/5 /					

APPENDIX 3 (Cont'd)

Date Feeding	Date Collection	Dried Bracken Consumed gm.	Urine Daily Excretion cc.	Live Weight lb.	Temperature °F
		Sheep G - Dried bracken (Cont'd)			
17/5	18/5/50	920	1430		
18/5	19/5	800	2200		103.6
19/5	20/5	480	2130		
20/5	21/5	135	1850	101.0	106.7 / 106.6
21/5	22/5	180	2060		105.8 / 106.9
22/5	23/5	nil	1675		105.4 / 106.4
23/5	24/5	nil	1550		105.4 / 106.7
24/5	25/5	nil	240	96.0	105.6
		Sheep died 24/5/50			
Total		42,861			

APPENDIX 4

NaCN Dosing and Alcoholic bracken extract feeding

Daily record sheet

Date Feeding	Date Collection	Hay Consumed lb.	Urine Daily Excretion cc.	Temperature F.
		Bullock 5 - Hay fed		
19/2	20/2/50	11.5	1250	
20/2	21/2	9.5	1660	101.4
21/2	22/2	11.0	1650	
22/2	23/2	11.5	1510	
23/2	24/2	9.75	1160	
24/2	25/2	11.0	1650	101.8
25/2	26/2	7.75	2300	
26/2	27/2	10.0	1820	
27/2	28/2	11.75	2278	
28/2	1/3	12.25	2952	101.4
1/3	2/3	10.75	2860	
2/3	3/3	12.50	3400	
3/3	4/3	12.75	3380	
4/3	5/3	13.50	2910	101.4
5/3	6/3	11.75	2480	
6/3	7/3	11.75	2800	
7/3	8/3	12.0	3125	
8/3	9/3	11.0	1300	101.6
9/ /				

NaCN Dosing and Alcoholic bracken extract feeding

Daily record sheet

Date Feeding	Collection	Hay Consumed lb.	Urine Daily Excretion cc.	Temperature °F.
		Bullock 5 - Hay fed (contd.)		
9/3	10/3/50	13.25	2720	
10/3	11/3	13.50	2870	
11/3	12/3	12.25	3100	
12/3	13/3	13.50	2370	101.6
13/3	14/3	11.50	3280	
14/3	15/3	13.50	2650	

Date		Hay Consumed lb.	HCN Consumed Per Day mg.	Urine Daily Excretion cc.	Temperature °F.
Feeding	Collection				
		Bullock 5			
		89.35 mg. HCN absorbed in 100 gm. grass cubes fed per day			
15/3	- 16/3/50	14.25	89.35	2750	101.5
16/3	- 17/3	13.50	"	2850	102.0
17/3	- 18/3	12.25	"	2610	102.0
18/3	- 19/3	12.75	"	2690	101.4
19/3	- 20/3	13.00	"	2970	101.6
20/3	- 21/3	14.00	"	2820	101.4
21/3	- 22/3	14.00	"	2700	101.4
22/3	- 23/3	14.00	"	2810	101.4
23/3	- 24/3	14.50	"	3190	101.4
24/3	- 25/3	14.75	"	2850	101.4
25/3	- 26/3	14.5	"	2870	101.3
26/3	- 27/3	14.25	"	3280	101.2
27/3	- 28/3	15.00	"	2710	101.4
28/3	- 29/3	14.25	"	3050	101.2
29/3	- 30/3	14.25	"	2850	101.4
30/3	- 31/3	14.00	"	3070	101.2
31/3	- 1/4	13.75	"	2580	101.4
1/4	- 2/4	14.25	"	3130	101.3
2/4	- 3/4	13.00	"	2590	101.4
3/4	- 4/4	13.50	"	2500	101.4
4/4	/				

Date Feeding	Date Collection	Hay Consumed lb.	HCN Consumed Per Day mg.	Urine Daily Excretion cc.	Temperature °F.
		Bullock 5			
89.35 mg. HCN absorbed in 100 gm. grass cubes fed per day (Cont'd)					
4/4	5/4/50	11.75	89.35	2100	101.3
5/4	6/4	13.50	"	2320	100.6
6/4	7/4	12.50	"	2130	100.8
7/4	8/4	12.75	"	2310	101.2
8/4	9/4	14.0	"	2960	100.4
9/4	10/4	13.0	"	2400	101.0
10/4	11/4	12.0	"	2470	102.0
11/4	12/4	13.25	"	2820	102.0
Total HCN dosed			2.502 gm.		
HCN increased to 134.03 mg. in 100 gm. grass cubes					
12/4	13/4	12.0	134.03	3380	102.0
13/4	14/4	13.0	"	2840	102.0
14/4	15/4	Hay now fed Ad. Lib.	"	2480	101.8
15/4	16/4	Hay Ad. Lib.	"	2450	101.7
16/4	17/4	"	"	2360	101.4
17/4	18/4	"	"	3120	101.4
18/4	19/4	"	"	2550	101.6
19/4	20/4	"	"	2130	101.3
20/4	21/4	"	"	2480	100.2
21/4 /					

Date Feeding	Date Collection	Hay Consumed lb.	HCN consumed Per Day mg.	Urine Daily Excretion cc.	Temperature °F.
		Bullock 5			
		HCN increased to 134.03 mg. in 100 gm. grass cubes (Cont'd)			
21/4 -	22/4/50	Hay Ad. Lib.	134.03	2630	101.2
22/4 -	23/4	"	"	2400	101.0
23/4 -	24/4	"	"	2820	100.8
24/4 -	25/4	"	"	2480	101.0
25/4 -	26/4	"	"	2310	100.4
26/4 -	27/4	"	"	2420	101.0
27/4 -	28/4	"	"	2430	100.5
28/4 -	29/4	"	"	3150	100.8
29/4 -	30/4	"	"	2790	101.0
30/4 -	1/5	"	"	2290	100.5
1/5 -	2/5	"	"	2820	101.0
2/5 -	3/5	"	"	2200	101.0
3/5 -	4/5	"	"	710*	101.2
4/5 -	5/5	"	"	3210	100.8
5/5 -	6/5	"	"	2790	100.8
6/5 -	7/5	"	"	3000	101.4
7/5 -	8/5	"	"	2470	101.4
8/5 -	9/5	"	"	2760	101.2
9/5 -	10/5	"	"	2940	101.2
10/5 -	11/5	"	"	2750	101.4
11/5 /					

* Loss

Date Feeding	Collection	Hay Consumed lb.	HCN consumed per Day mg.	Urine Daily Excretion cc.	Temperature °F.
		Bullock 5			
HCN increased to 134.03 mg. in 100 gm. grass cubes (Cont'd)					
11/5	- 12/5/50	Hay Ad. Lib.	134.03	2950	101.4
12/5	- 13/5	"	"	2850	101.0
Total HCN dosed			4.155 gm.		

Grand total HCN dosed 6.657 gm.

APPENDIX 4 (Cont'd)

Date Feeding	Date Collection	Hay Consumed lb.	Urine Daily Excretion cc.	Temperature °F.
		Bullock 5 (Cont'd)		
		Hay		
23/6 -	24/6/50	Ad. Lib.	-	100.7
24/6 -	25/6	"	3200	
25/6 -	26/6	"	2540	
26/6 -	27/6	"	2830	
		Hay treated with alcoholic bracken extract		
27/6 -	28/6	10	6450	100.8
28/6 -	29/6	)	3700	
		)		
29/6 -	30/6	)	3430	
		) 14.75		
30/6 -	1/7	)	4740	98.8
		)		
1/7 -	2/7	)	6240	99.5
2/7 -	3/7	6	6370	100.2
3/7 -	4/7	6.75	4240	100.0
4/7 -	5/7	9.50	4820	100.2
5/7 -	6/7	9.25	5320	99.5
6/7 -	7/7	10.25	4670	99.8
7/7 -	8/7	7.75	3970	99.6
8/7 -	9/7	7.25	3435	100.5
9/7 -	10/7	8.50	3316	99.6
Total		90 lb.		

Date Feeding	Date Collection	Hay Fed gm.	Urine Daily Excretion cc.	Live Weight lb.	Temperature °F.
			Sheep H (control)		
2/4 -	3/4/50	Ad. Lib.	350		
3/4 -	4/4	"	360		
4/4 -	5/4	"	440		
5/4 -	6/4	"	450	95.25	
6/4 -	7/4	"	515		
7/4 -	8/4	"	640		103.8
8/4 -	9/4	"	650		
9/4 -	10/4	"	670	100.5	
10/4 -	11/4	"	530		
11/4 -	12/4	"	620		103.7
12/4 -	13/4	"	600		
13/4 -	14/4	"	710	101.25	
14/4 -	15/4	"	700		
15/4 -	16/4	"	610		
16/4 -	17/4	"	1150		103.6
17/4 -	18/4	"	620		
18/4 -	19/4	"	600	102.0	
19/4 -	20/4	"	640		
20/4 -	21/4	"	610		103.6
21/4 -	22/4	"	580		
22/4 /					

APPENDIX 4 (Cont'd)

Date Feeding	Date Collection	Hay Fed gm.	Urine Daily Excretion cc.	Live Weight lb.	Temperature °F.
		Sheep H (control) (Cont'd)			
22/4	23/4/50	Ad. Lib.	530	103.0	
23/4	24/4	"	540		
24/4	25/4	"	510		103.4
25/4	26/4	"	630		
26/4	27/4	"	710	103.0	
27/4	28/4	"	840		
28/4	29/4	"	735		103.2
29/4	30/4	"	750		
30/4	1/5	"	915	102.5	
1/5	2/5	"	790		
2/5	3/5	"	740		103.1
3/5	4/5	"	700		
4/5	5/5	"	700	105.5	
5/5	6/5	"	720		
6/5	7/5	"	710		102.2
7/5	8/5	"	580		
8/5	9/5	"	670	106.75	
9/5	10/5	"	710		
10/5	11/5	"	570		103.8
11/5	12/5	"	760		
12/5	13/5	"	560	108.0	
13/5	14/5	"	700		
14/5	15/5	"	725		103.2

APPENDIX 4 (Cont'd)

Date Feeding	Date Collection	Hay Fed gm.	NaCN Drenched mg.	Urine Daily Excretion cc.	Live Weight lb.	Temperature °F.
			Sheep H (control) (Cont'd)			
			Hay + 54.108 mg. HCN drenched			
15/5	16/5/50	Ad.Lib.	54.108	700		
16/5	17/5	"	"	630	106.0	
17/5	18/5	"	"	620		
18/5	19/5	"	"	595		103.5
19/5	20/5	"	"	610		
20/5	21/5	"	"	600	108.5	
21/5	22/5	"	"	690		
22/5	23/5	"	"	750		103.4
23/5	24/5	"	"	660		
24/5	25/5	"	"	670	108.0	
25/5	26/5	"	"	670		
26/5	27/5	"	"	680		103.1
27/5	28/5	"	"	675		
28/5	29/5	"	"	890	106.0	
29/5	30/5	"	"	710		
30/5	31/5	"	"	725		103.2
31/5	1/6	"	"	730		
1/6	2/6	"	"	680	104.5	
2/6	3/6	"	"	760		
3/6	4/6	"	"	750		103.8
4/6	/					

Date Feeding	Date Collection	Hay Fed gm.	NaCN Drenched mg.	Urine Daily Excretion cc.	Live Weight lb.	Temperature °F.
		Sheep H (control) (Cont'd)				
		Hay + 54.108 mg. HCN drenched				
4/6 -	5/6/50	Ad.Lib.	54.108	920		
5/6 -	6/6	"	"	890	101.5	
6/6 -	7/6	"	"	700		
7/6 -	8/6	"	"	780		104.0
8/6 -	9/6	"	"	800		
9/6 -	10/6	"	"	780	103.5	
10/6 -	11/6	"	"	755		
11/6 -	12/6	"	"	710		103.5
12/6 -	13/6	"	"	660		
13/6 -	14/6	"	"	730	102.0	
14/6 -	15/6	"	"	710		
15/6 -	16/6	"	"	660		103.0
16/6 -	17/6	"	"	620		
17/6 -	18/6	"	"	690	96.0	
18/6 -	19/6	"	"	630		
19/6 -	20/6	"	"	600		102.0
20/6 -	21/6	"	"	650		
21/6 -	22/6	"	"	700	97.5	
22/6 /						

APPENDIX 4 (Cont'd)

Date Feeding	Date Collection	Hay Fed gm.	NaCN Drenched mg.	Urine Daily Excretion cc.	Live Weight lb.	Temperature °F.
		Sheep H (control) (Cont'd)				
		Hay + 54.108 mg. HCN drenched				
22/6 -	23/6/50	Ad.Lib.	54.108	720		
23/6 -	24/6	"	"	730		101.6

Total HCN given = 2.164 gm. HCN

24/6-31/7/50 Total HCN dosed in 38 days = 2.056 gm. HCN

∴ Grand total HCN dosed = 4.220 gm. HCN

APPENDIX 5

HCN Daily variations in Bracken

Date	Mg HCN/100 G Dry Matter	Date	Mg HCN/100 G Dry Matter
	Bullock I - Fresh bracken fed 18/6/49 - 16/7/49 & 28/7/49 & 11/8/49		
18/6/49	1.605	4/7/49	1.835
19/6	1.903	5/7	2.229
20/6	1.815	6/7	2.708
21/6	7.120	7/7	1.944
22/6	8.820	8/7	2.752
23/6	3.080	9/7	1.176
24/6	3.295	10/7	2.047
25/6	5.300	11/7	1.128
26/6	3.698	12/7	1.385
27/6	1.923	13/7	0.970
28/6	2.065	14/7	7.308
29/6	3.144	15/7	4.787
30/6	7.218	16/7	5.232
		Mean value	3.350
1/7	1.600	28/7 (Mature	0.248
		(Green	
2/7	3.297	(Young	13.180
		(Shoots	
3/7	5.425	11/8 (Mature	1.566
		(Green	
		(Young	15.722
		(Shoots	

Bullock 4 - Fresh bracken fed
2nd Experiment - 18/8/49 - 21/10/49

Date	Bracken fed HCN/100g. D.M. mg.	Date	Bracken fed HCN/100g. D.M. mg.
18/8/49	3.57	7/9/49	1.65
19/8	2.39	8/9	2.96
20/8	3.02	9/9	2.35
21/8	4.06	10/9	0.93
22/8	2.84	11/9	1.36
23/8	4.65	12/9	0.10
24/8	2.87	13/9	0.96
25/8	4.13	14/9	1.84
26/8	2.88	15/9	0.32
27/8	3.69	16/9	0.85
28/8	1.90	17/9	0.50
29/8	2.70	18/9	0.11
30/8	3.66	19/9	0.16
31/8	2.44	20/9	0.42
1/9	1.37	21/9	0.92
2/9	3.44	22/9	0.66
3/9	2.35	23/9	0.90
4/9	2.33	24/9	0.34
5/9	1.75	25/9	0.04
6/9	0.31	26/9	0.08
		27/9 /	

Date	Bracken fed HCN/100g. D.M. mg.	Date	Bracken fed HCN/100g. D.M. mg.
	Bullock 4 - Fresh bracken fed 2nd Experiment - 18/8/49 - 21/10/49 (Cont'd)		
27/9/49	0.02	3/10/49	0.30
28/9	0.03	4/10	0.10
29/9	0.02	5/10	0.03
30/9	0.02	6/10	0.04
1/10	0.04	7/10	0.03
2/10	0.11	8/10	0.07
		Mean value	1.45
	Young second growth bracken		
9/10	9.36	16/10	7.94
10/10	10.12	17/10	6.06
11/10	8.63	18/10	10.02
12/10	6.16	19/10	5.18
13/10	8.95	20/10	6.56
14/10	6.38	21/10	5.34
15/10	5.19	Mean value	7.35

APPENDIX 6

HCN and HCNS in urine samples

Date	Urine Volume cc.	mg.HCN in Total Urine	mg.HCNS as HCN in Total Urine	Total HCN in Urine mg.	mg. HCN in Urine Daily Average	mg. HCN in Urine per 100cc.
		Sheep G - (14/3/49 - 21/5/50)				
		Hay fed				
14/3/50	590	0.055	0.836	0.891	0.891	0.152
15/3	790	0.049	1.793	1.842	1.842	0.233
16/3	750	0.070	1.170	1.240	1.240	0.165
		Mean value			1.324	0.183
		Dried bracken fed				
17-19/3	4400	0.273	9.988	10.261	3.420	0.233
24-27/3	2520	0.156	8.652	8.808	2.202	0.350
1- 4/4	2420	0.300	9.103	9.403	2.351	0.389
9-12/4	4050	0.126	9.194	9.320	2.330	0.230
14-19/4	6105	0.199	16.026	16.225	2.704	0.265
21-26/4	8210	0.511	18.634	19.145	3.191	0.233
28- 3/5	8640	0.268	25.738	26.006	4.334	0.301
5-10/5	8870	0.550	12.582	13.132	2.188	0.149
13-17/5	7300	0.453	15.513	15.966	3.193	0.218
19-21/5	6180	0.192	14.460	14.652	4.884	0.237
		Mean value			3.080	0.260

Date	Urine Volume cc.	mg.HCN in Total Urine	mg.HCNS as HCN	Total HCN in Urine mg.	mg. HCN in Urine Daily Average	mg. HCN in Urine per 100cc.
			Sheep C - (14/3/50 - 7/6/50)			
			Hay fed			
14/3/50	570	0.106	2.587	2.593	2.593	0.455
15/3	700	0.130	2.383	2.513	2.513	0.359
		Mean value			2.553	0.408
			Dried bracken fed			
9-12/4	3250	0.101	8.537	8.638	2.159	0.265
14-19/4	4515	0.560	8.967	9.527	1.588	0.211
21-26/4	7060	1.094	17.026	18.120	3.020	0.257
28/4-3/5	6345	0.393	14.401	14.794	2.466	0.233
5/5-10/5	7935	1.230	11.256	12.486	2.081	0.158
		Mean value			2.263	0.225
			Extracted bracken cubes fed			
13-17/5	10,260	1.272	17.467	18.739	3.748	0.183
19-28/5	28,420	0.881	52.395	53.276	5.328	0.187
29-31/5	11,200	1.042	16.708	17.750	5.917	0.158
2-7/6	24,625	0.763	36.735	37.498	6.249	0.152
		Mean value			5.310	0.170

Date	Urine Volume cc.	mg.HCN in Total Urine	mg.HCNS as HCN in Urine cc.	Total HCN in Urine mg.	mg. HCN in Urine Daily Average	mg. HCN in Urine per 100cc.
Sheep D - (14/3/50 - 7/6/50)						
		Hay fed				
14/3/50	720	0.179	2.556	2.735	2.735	0.380
15/3	760	0.212	2265	2.477	2.477	0.326
16/3	850	0.264	3.018	3.282	3.282	0.386
		Mean value			2.831	0.364
		Dried bracken fed				
17-19/3	3185	0.395	6.100	6.495	2.165	0.204
24-27/3	1450	0.315	2.057	2.372	0.593	0.163
1-4/4	940	0.058	2.002	2.060	0.515	0.213
9-12/4	1350	0.042	1.917	1.959	0.489	0.145
14-19/4	3940	0.366	3.349	3.715	0.619	0.094
21-26/4	5055	0.313	6.454	6.767	1.128	0.134
28-3/5	5415	0.336	6.913	7.249	1.208	0.134
5-10/5	5073	0.472	9.319	9.791	1.632	0.193
		Mean value			1.044	0.160
		Whole bracken cubes fed				
12-17/5	8605	0.534	12.218	12.752	2.125	0.148
19-28/5	15070	0.934	23.521	24.455	2.352	0.162
29-31/5	3415	0.212	6.315	6.527	2.176	0.191
2-7/6	12240	0.759	22.572	23.331	3.889	0.190
		Mean value			2.635	0.173

Date	Urine Volume cc.	mg.HCN in Total Urine	mg.HCNS as HCN in Total Urine	Total HCN in Urine mg.	mg. HCN in Urine Daily Average	mg. HCN in Urine per 100cc.
		Bullock 5 - (10/2/50 - 27/2/50)				
		Control hay fed				
10/2/50	2815	0.394	20.940	21.334	21.334	0.757
11/2	3270	0.654	25.513	26.167	26.167	0.800
12/2	4065	0.366	27.679	28.045	28.045	0.689
		Mean value			25.182	0.749
		Starvation				
13/2	5030	0.603	16.768	17.371	17.371	0.345
14/2	6060	0.376	15.474	15.850	15.850	0.261
15/2	6300	0.586	12.079	12.665	12.665	0.201
16/2	5470	0.678	8.535	9.213	9.213	0.169
		Small feed - Hay				
17/2	1190	0.111	2.195	2.306	2.306	0.193
18/2	1200	0.112	2.894	3.006	3.006	0.251
19/2	1180	0.110	3.850	3.960	3.960	0.335
		Full feed - Hay				
20/2	1250	0.078	3.106	3.184	3.184	0.252
21/2	1660	0.103	3.871	3.974	3.974	0.239
22/2	1650	0.153	2.999	3.152	3.152	0.191
23/2	1510	0.187	1.824	2.011	2.011	0.133
24/2	1160	0.144	3.705	3.849	3.849	0.332
25/2	1650	0.153	6.086	6.239	6.239	0.378
26/2	2300	0.285	7.346	7.631	7.631	0.332
27/2	1820	0.169	6.846	7.015	7.015	0.385

Date	Urine Volume cc.	mg.HCN in Total Urine	mg.HCNS as HCN in Total Urine	Total HCN in Urine mg.	mg. HCN in Urine Daily Average	mg. HCN in Urine per 100cc.
		Bullock 5 (Cont'd) - (13/3/50 - 12/5/50)				
		Control hay fed				
13/3/50	2370	0.220	11.095	11.315	11.315	0.477
15/3	2650	0.164	14.474	14.638	14.638	0.552
		Mean value			12.977	0.515
		89.35 mg. HCN + hay fed				
16-19/3	8210	0.764	41.344	42.108	14.036	0.513
24-27/3	12190	3.779	61.387	65.166	16.292	0.534
1-4/4	10800	3.348	55.919	59.267	14.817	0.549
9-12/4	10650	1.320	42.280	43.600	10.900	0.409
		Mean value			14.011	0.501
		134.030 mg. HCN + hay fed				
14-19/4	15800	6.367	70.942	77.309	12.885	0.489
21-26/4	15120	1.406	64.346	65.752	10.959	0.435
28/4-3/5	15680	2.916	64.505	67.421	11.237	0.430
6-9/5	11020	2.733	38.300	41.033	10.258	0.372
10/5	2940	0.456	9.175	9.631	9.631	0.327
11/5	2750	0.171	8.494	8.665	8.665	0.315
12/5	2950	0.640	11.272	11.912	11.912	0.404
		Mean value			10.792	0.396

Date	Urine Volume cc.	mg.HCN in Total Urine	mg. HCNS as HCN	Total HCN in Urine mg.	mg. HCN in Urine Daily Average	mg. HCN in Urine per 100cc.
		Bullock 5 (Cont'd) - (25/6/50 - 10/7/50)				
		Hay fed				
25-26/6/50	5740	0.356	13.437	13.793	6.897	0.243
		Alcoholic bracken extract mixed with hay				
27/6-1/7	21150	0.656	48.011	48.667	9.733	0.230
2-6/7	26990	0.837	61.270	62.107	12.421	0.230
7-10/7	15391	0.477	37.120	37.597	9.399	0.244
		Mean value			10.518	0.235

Date	Urine Volume cc.	mg.HCN in Total Urine	mg. HCNS as HCN	Total HCN in Urine mg.	mg. HCN in Urine Daily Average	mg. HCN in Urine per 100cc.
			Sheep H - (14/3/50 - 7/6/50)			
			Control hay fed			
14/3/50	710	0.044	1.813	1.857	1.857	0.262
15/3	720	0.045	2.451	2.496	2.496	0.347
14-19/4	4390	0.544	17.437	17.981	2.997	0.410
13-15/5	1985	0.246	6.899	7.145	2.382	0.360
		Mean value			2.433	0.345
			54.108 mg. HCN + hay fed			
16/5	700	0.217	2.631	2.848	2.848	0.407
17-28/5 Except 22/5	7160	0.666	14.352	15.018	1.365	0.210
29-31/5	2325	0.505	8.100	8.605	2.868	0.370
2-7/6	4700	0.290	13.334	13.624	2.271	0.290
		Mean value			2.338	0.319

APPENDIX 7

HCN and HCNS in blood samples

No.	Date	Plasma		Serum	
		mg.HCN/100cc.	mg/HCNS/100cc.	mg.HCN/100cc.	mg.HCNS/100cc.
		Bullock 1 - (18/6/49 - 17/7/49)			
		Hay fed			
1	9/6/49	nil	0.149	-	-
2	15/6	0.006	0.025	0.006	0.037
	Mean value	0.003	0.087	0.006	0.037
		Fresh bracken fed			
3	29/6	nil	0.595	-	-
4	14/7	0.012	0.471	nil	0.347
5	17/7	0.012	0.508	0.025	0.496
	Mean value	0.008	0.525	0.013	0.422

No.	Date	Plasma mg.HCN/100cc.	Plasma mg.HCNS/100cc.	Serum mg.HCN/100cc.	Serum mg.HCNS/100cc.
		Sheep C - (18/6/49 - 17/7/49)			
		Hay fed			
1	9/6/49	nil	nil	nil	0.099
2	15/6	0.006	0.174	0.006	0.149
	Mean value	0.003	0.087	0.003	0.124
		Fresh bracken fed			
3	29/6	nil	0.112	0.006	0.124
4	14/7	0.012	0.198	0.012	0.149
5	17/7	0.025	0.198	0.006	0.211
	Mean value	0.012	0.169	0.008	0.161

No.	Date	Plasma mg.HCN/100cc.	Plasma mg.HCNS/100cc.	Serum mg.HCN/100cc.	Serum mg.HCNS/100cc.
		Bullock 4 - (18/8/49 - 24/10/49)			
		Hay fed			
1	11/8/49	nil	0.248	-	-
2	17/8	nil	0.298	-	-
	Mean value	nil	0.273	-	-
		Fresh bracken fed			
3	30/8	0.006	0.446	-	-
4	6/9	nil	0.322	-	-
5	13/9	nil	0.409	-	-
6	20/9	nil	0.496	-	-
7	27/9	nil	0.372	nil	0.310
8	4/10	nil	0.446	0.025	0.360
9	14/10	nil	0.595	-	-
10	22/10	0.019	0.347	-	-
11	24/10	0.037	0.446	nil	0.719
	Mean value	0.007	0.431	0.008	0.463

No.	Date	Plasma		Serum	
		mg.HCN/100cc.	mg.HCNS/100cc.	mg.HCN/100cc.	mg.HCNS/100c
		Sheep E - (18/8/49 - 21/10/49)			
		Hay fed			
1	3/8/49	nil	0.087	0.006	0.198
2	17/8	nil	0.099	0.006	0.149
	Mean value	nil	0.093	0.006	0.174
		Fresh bracken fed			
3	30/8	0.006	0.074	-	-
4	6/9	nil	0.050	nil	0.050
5	13/9	nil	0.037	nil	0.198
6	27/9	nil	0.074	nil	0.124
7	4/10	0.012	0.050	0.012	0.050
8	14/10	nil	0.149	0.012	0.050
9	21/10	nil	0.248	0.012	0.087
	Mean value	0.003	0.098	0.006	0.093

No.	Date	Plasma		Serum	
		mg.HCN/100cc.	mg.HCNS/100cc.	mg.HCN/100cc.	mg.HCNS/100cc.
		Sheep F - (18/8/49 - 23/10/49)			
		Hay Fed			
1	3/8/49	nil	0.087	nil	0.062
2	17/8	nil	0.099	0.006	0.149
	Mean value	nil	0.093	0.003	0.106
		Fresh bracken fed			
3	30/8	0.006	0.174	nil	0.149
4	6/9	nil	0.136	nil	0.087
5	13/9	0.006	0.136	nil	0.087
6	27/9	nil	0.186	nil	0.149
7	4/10	0.006	0.149	0.006	0.186
8	14/10	0.006	0.186	0.012	0.186
9	23/10	0.006	0.186	0.006	0.595
	Mean value	0.004	0.156	0.003	0.206

No.	Date	Plasma mg.HCN/100cc.	Plasma mg.HCNS/100cc.	Serum mg.HCN/100cc.	Serum mg.HCNS/100cc.
		Sheep C & D controls - (18/8/49 - 24/10/49)			
			Hay fed		
			Sheep C		
1	3/8/49	0.006	0.136	0.012	0.310
2	17/8	nil	0.049	nil	0.049
3	30/8	nil	0.049	-	-
4	6/9	nil	0.049	-	-
5	13/9	nil	0.037	nil	0.149
6	27/9	nil	0.049	nil	0.087
	Mean value	0.001	0.062	0.003	0.149
			Sheep D		
7	3/8	0.006	0.099	0.006	0.198
8	17/8	nil	0.037	nil	0.037
9	4/10	0.012	0.124	0.006	0.112
10	14/10	nil	0.050	0.012	0.050
	Mean value	0.005	0.077	0.006	0.099

Date	Feed	Blood plasma mg.HCN/100cc.	Blood plasma mg.HCNS/100cc.
	Sheep G		
5/3/50	Hay	nil	0.050
10/3	"	nil	0.050
Mean value		nil	0.050
3/4	Dried bracken	nil	0.050
	Sheep D		
5/3	Hay	0.025	0.025
10/3	"	0.012	0.025
Mean value		0.019	0.025
3/4	Dried bracken	nil	0.087
30/5	Whole bracken cubes	0.025	0.198
	Sheep C		
22/2	Hay	0.012	0.050
5/3	"	nil	0.050
10/3	"	nil	0.087
3/4	"	nil	0.025
Mean value		0.003	0.053
30/5	Extracted bracken cubes	0.012	0.149

Date	Feed	Blood plasma mg.HCN/100cc.	Blood plasma mg.HCNS/100cc.
	Bullock 5		
10/3/50	Hay	nil	0.124
13/3	"	0.012	0.124
Mean value		0.006	0.124
11/4	NaCN + hay	0.012	0.332
	Sheep H - Control		
5/3	Hay control	0.012	0.050
10/3	" "	0.012	0.025
3/4	" "	nil	0.025
Mean value		0.008	0.033

APPENDIX 8

Urine Alkalinity

No.	Date	Urine daily excretion cc.	Normality of urine	M.Eq. Alkali excreted	
		Bullock 1			
		hay fed			
1	17/6/49	5800	0.378	2192	
2	18/6	4680	0.410	1919	
	Mean value			2056	
		Fresh bracken fed			
3	19-22/6	5673	0.732	4153	
4	23-26/6	5495	0.672	3693	
5	27-30/6	5945	0.516	3068	
6	1-4/7	5099	0.610	3110	
7	5-8/7	5295	0.536	2838	
8	9-12/7	5413	0.443	2398	
9	13-16/7	3825	0.878	3358	
10	16-17/7 (overnight)	3250	1.129	3669	
11	17/7 (9.30-1.30p.m.)	730	0.896	654	
12	17/7 (1.30-5.30p.m.)	820	0.902	740	2780
13	17-18/7 (overnight)	1650	0.840	1386	
14	18/7 (ex bladder)	750	0.713	535	
	Mean value (4 - 14)			3114	

No.	Date	Urine daily excretion cc.	Normality of urine	M.Eq. Alkali excreted
		Sheep C		
		hay fed		
1	16/6/49	860	0.368	316
2	17/6	700	0.166	116
		Mean value		216
		Fresh bracken fed		
3	19-22/6	947	0.583	552
4	23-26/6	493	0.740	365
5	27-30/6	558	0.532	297
6	1-4/7	308	0.851	262
7	5-8/7	285	0.942	268
8	9-12/7	418	0.616	257
9	13-16/7	364	0.700	255
		Mean value (4 - 9)		284

No.	Date	Urine daily excretion cc.	Normality of urine	M.Eq. Alkali excreted
		Bullock 4		
		hay fed		
1	13-14/8/49	1228	0.670	823
2	15-16/8	1545	0.378	584
3	17-18/8	1515	0.473	717
		Mean value		708

No.	Date	Urine daily excretion cc.	Normality of urine	M.Eq. Alkali excreted
		Bullock 4		
		Fresh bracken fed		
1	19-22/8/49	2230	0.699	1559
2	23-26/8	1828	0.818	1495
3	27-30/8	1623	0.950	1542
4	31/8-3/9	1190	1.223	1455
5	4-7/9	1335	1.023	1366
6	8-9/9	1380	0.737	1017
7	10/9	1340	0.713	955
8	11/9	950	0.386	367
9	12-15/9	1358	0.813	1104
10	16-19/9	1500	0.821	1232
11	20-23/9	1446	0.956	1382
12	24-27/9	1305	0.964	1258
13	28/9-1/10	1386	0.689	955
14	2-5/10	1393	0.902	1256
15	6-9/10	1170	0.910	1065
16	10-13/10	1378	0.786	1083
17	14-17/10	1558	0.797	1242
18	18-21/10	1538	1.053	1620
19	22/10	1460	1.291	1885
	Mean value (2 - 19)			1238

No.	Date	Urine daily excretion cc.	Normality of urine	M.Eq. Alkali excreted
		Sheep E		
		hay fed		
1	18/8/49	950	0.405	385
		Fresh bracken fed		
2	19-22/8	968	0.637	617
3	27-30/8	468	0.583	273
4	4-7/9	373	0.702	262
5	12-15/9	238	0.869	207
6	19-23/9	193	0.890	172
7	28/9-1/10	280	0.416	116
8	6-9/10	323	0.475	153
9	14-17/10	555	0.356	198
		Mean value (3 - 9)		198

No.	Date	Urine daily excretion cc.	Normality of urine	M.Eq. Alkali excreted
		Sheep F		
		hay fed		
1	18/8/49	1290	0.416	537
		Fresh bracken fed		
2	19-22/8	2533	0.308	780
3	27-30/8	578	0.626	362
4	4-7/9	743	0.621	461
5	12-15/9	651	0.551	359
6	19-23/9	639	0.254	162
7	28/9-1/10	740	0.216	160
8	6-9/10	516	0.265	137
9	14-17/10	1059	0.329	348
10	22-23/10	1040	0.243	253
		Mean value (3 - 10)		280

APPENDIX 8 (Cont'd)

No.	Date	Urine daily excretion cc.	Normality of urine	M.Eq. Alkali excreted
		Sheep C		
		hay fed		
1	14/3/50	570	0.140	80
2	15/3	700	0.145	102
3	18/3	-	0.142	-
4	19/3	-	0.187	-
5	28/3	650	0.140	91
		Mean value (1 - 5)		91
		Starvation		
6	29/3	620	0.231	143
7	30/3	990	0.091	90
8	31/3	1470	0.051	75
9	1/4	1310	0.038	50
10	2/4	710	0.038	27
11	3/4	1570	0.028	43
		Mean value (6 - 11)		71

No.	Date	Urine daily excretion cc.	Normality of urine	M.Eq. Alkali excreted
		Sheep D		
		hay fed		
1	14/3/50	720	0.175	126
2	15/3	760	0.199	151
3	16/3	850	0.223	190
		Mean value		156
		Dried bracken fed		
4	17/3	915	0.188	172
5	18/3	1200	0.121	145
6	19/3	1070	0.113	121
7	24/3	320	0.109	34.9
8	25/3	290	0.125	36.3
9	26/3	310	0.133	41.2
10	27/3	530	0.107	56.7
11	1/4	220	0.090	19.8
12	2/4	210	0.101	21.1
13	3/4	270	0.139	36.1
14	4/4	240	0.101	24.1
		Mean value (5 - 14)		54

No.	Date	Urine daily excretion cc.	Normality of urine	M.Eq. Alkali excreted
		Sheep G		
		hay fed		
1	14/3/50	590	0.118	70
2	15/3	790	0.142	112
3	16/3	750	0.215	161
		Mean value		114
		Dried bracken fed		
4	17/3	1500	0.172	258
5	18/3	1850	0.152	281
6	19/3	1060	0.117	124
7	24/3	530	0.078	41.3
8	25/3	800	0.073	58.4
9	26/3	660	0.059	38.9
10	27/3	530	0.070	37.1
11	1/4	650	0.093	60.1
12	2/4	520	0.081	41.9
13	3/4	600	0.088	52.8
14	4/4	600	0.082	49.2
		Mean value (5 - 14)		78.5

No.	Date	Urine daily excretion cc.	Normality of urine	M.Eq. Alkali excreted
		Bullock 5		
		hay fed		
1	13/3/50	2370	0.290	687
2	15/3	2650	0.228	604
		Mean value		646
		Hay + NaCN fed		
3	16/3	2750	0.263	723
4	17/3	2850	0.320	912
5	18/3	2610	0.275	718
6	19/3	2690	0.286	769
7	24/3	3190	0.265	845
8	25/3	2850	0.254	724
9	26/3	2870	0.271	778
10	27/3	3280	0.303	994
11	1/4	2580	0.276	711
12	2/4	3130	0.270	845
13	3/4	2590	0.265	636
14	4/4	2500	0.228	569
		Mean value (4 - 14)		773

APPENDIX 9

Fresh bracken - 2nd experiment

Vitamin B_1 in urine

Vitamin B_1 µg/1 ml. urine

Date	Bullock 4	Sheep E	Sheep F	Sheep D hay fed
17-18/8/49 (normal)	1.30	-	-	-
	Fresh bracken fed			
1/9	0.42	-	-	-
2-3/9	0.55	0.25	nil	-
7-8/9	0.15	-	-	-
13-14/9	0.10	0.05	0.05	-
18-19/9	0.26	0.15	nil	-
27/9	0.06	-	-	-
16/10	-	-	nil	2.05
18/10	nil	nil	nil	1.35
20/10	nil	nil	nil	-
21/10 (4-6p.m.)	(310.2 *	0.40 *	-	-
(6-9p.m.)	(253	- died	-	-
22/10	40	-	nil	-
23/10	63	-	0.64 *	-
24/10	10 died	-	died	-

* after injection of vitamin B_1 intravenously.

APPENDIX 9 (Cont'd)

Pyruvic acid in urine and blood

Urine samples				Blood samples	
		Pyruvic acid mg.			
Date	Daily excretion cc.	per 100cc.	Total daily excretion	Date	Pyruvic acid mg/100cc.
		Bullock 4 - Bracken fed			
17-18/8/49 (normal)	1680	9.2	154.6	11/8/49	1.7
2-3/9	1240	5.9	73.2	17/8	2.0
13-14/9	1280	4.9	62.7	30/8	1.9
19-20/9	1424	5.2	74.0	6/9	1.4
18/10	1380	2.3	31.7	13/9	1.7
20/10	1635	3.1	50.7	20/9	2.9
21/10 (4-6p.m.)	64	3.4	2.2	26/9	3.2
21/10 (6-9p.m.)	110	4.5	5.0	4/10	1.1
22/10 (10a.m.)	55	4.0	2.2	14/10	1.7
		Died on 24/10/49			
24/10 urine ex bladder.	178	29.5	52.5	21/10	0.9
				24/10	8.5

Urine samples				Blood samples	
Date	Daily excretion cc.	Pyruvic acid mg. per 100cc.	Pyruvic acid mg. Total daily excretion	Date	Pyruvic acid mg/100cc.
		Sheep C control Hay fed			
17-18/8/49	744	14.0	104.1	11/8/49	0.95
2-3/9	740	6.7	49.6	17/8	1.1
13-14/9	650	5.5	35.8	30/8	1.5
19-20/9	730	8.0	58.0	6/9	1.1
				13/9	0.8
				20/9	-
				26/9	1.97
Average		8.55			1.06

Urine samples				Blood samples	
		Pyruvic acid mg.			
Date	Daily excretion cc.	per 100cc.	Total daily excretion	Date	Pyruvic acid mg/100cc.
		Sheep E - Bracken fed			
17-18/8/49 (normal)	950	7.2	68.4	11/8/49	1.3
2-3/9	350	4.5	15.8	17/8	1.5
13-14/9	260	5.5	14.3	30/8	0.95
19-20/9	190	7.0	13.3	6/9	2.0
20/10	530	3.9	20.7	13/9	2.99
21/10	-	5.3	-	20/9	2.5
				26/9	2.7
				4/10	1.8
				14/10	2.2
				21/10	1.5
		Sheep F - Bracken fed			
17-18/8/49 (normal)	1290	5.9	76.1	11/8/49	1.9
2-3/9	550	5.5	30.3	17/8	1.6
13-14/9	620	4.5	27.9	30/8	1.6
19-20/9	725	5.2	37.7	6/9	1.6
16/10	1045	4.5	47.0	13/9	1.3
20/10	1025	2.4	24.6	20/9	1.2
23/10	-	10.6	-	26/9	1.97
				4/10	1.1
				14/10	2.6
				22/10	4.2

APPENDIX 10

Dried bracken fed

Vitamin B_1 and pyruvic acid in urine

Date	Daily excretion cc.	Vitamin B_1		Pyruvic acid mg.	
		Urine samples			
		µg/1cc.	Daily excretion mg.	per 100cc.	Daily excretion
		Sheep C - hay fed			
6/3/50	385	0.245	0.094	-	-
7/3	500	0.359	0.180	-	-
8/3	550	0.444	0.244	-	-
20/3	640	-	-	2.507	16.045
21/3	655	-	-	2.434	15.943
22/3	620	-	-	2.159	13.385
23/3	690	-	-	2.654	18.313
	Mean value	0.349	0.173	2.438	15.922

Urine samples

Date	Daily excretion cc.	Vitamin B_1		Pyruvic acid mg.	
		µg/1cc.	Daily excretion mg.	per 100cc.	Daily excretion
		Sheep C - dried bracken fed			
5/4/50	950	0.168	0.160	1.171	11.125
6/4	670	0.202	0.135	1.940	11.998
7/4	550	0.139	0.076	1.080	5.940
8/4	1085	0.059	0.064	0.897	8.732
13/4	890	nil	nil	0.769	6.841
15/4	450	nil	nil	1.574	7.083
16/4	700	nil	nil	1.299	9.093
18/4	775	nil	nil	2.379	18.437
20/4	730	nil	nil	3.129	22.842
23/4	940	nil	nil	2.086	19.608
27/4	845	nil	nil	2.379	20.103
30/4	740	nil	nil	1.976	14.622
3/5	1200	nil	nil	-	-
4/5	1130	nil	nil	1.592	17.990
5/5	1890	nil	nil	-	-
6/5	1415	nil	nil	-	-
8/5	1140	nil	nil	-	-
9/5	1190	nil	nil	-	-
10/5	1040	nil	nil	-	-
11/5	1190	nil	nil	1.629	19.385
18/5	2320	nil	nil	1.976	45.843
1/6	3890	nil	nil	1.427	55.510
9/7	1800	nil	nil	0.952	17.136

Urine samples					
Date	Daily excretion cc.	Vitamin B_1		Pyruvic acid mg.	
		µg/1cc.	Daily excretion mg.	per 100cc.	Daily excretion
		Sheep D			
		Hay fed			
6/3/50	500	2.386	1.193	-	-
7/3	580	2.420	1.404	-	-
8/3	780	2.856	2.228	-	-
	Mean value	2.554	1.608		

APPENDIX 10 (Cont'd)

Urine samples

Date	Daily Excretion cc.	Vitamin B_1		Pyruvic acid mg.	
		µg/1cc.	Daily excretion mg.	per 100cc.	Daily excretion
		Sheep D - dried bracken fed			
20/3/50	600	1.045	0.627	4.136	24.816
21/3	520	1.188	0.618	5.517	28.688
22/3	480	0.733	0.352	7.082	33.994
23/3	459	0.728	0.334	8.015	36.789
28/3	350	0.192	0.067	8.107	28.375
29/3	250	0.101	0.025	7.988	19.750
30/3	290	0.112	0.032	9.333	27.066
31/3	250	0.166	0.042	10.834	27.095
5/4	345	0.193	0.067	5.673	19.572
6/4	270	nil	nil	6.167	16.651
7/4	265	nil	nil	1.519	4.025
8/4	330	nil	nil	3.825	12.623
13/4	520	nil	nil	2.928	15.226
20/4	740	nil	nil	2.361	17.471
27/4	890	nil	nil	3.129	27.851
4/5	910	nil	nil	2.050	18.655
11/5	870	nil	nil	2.379	20.697
18/5	1500	nil	nil	1.748	26.220
1/6	1010	nil	nil	1.903	19.220
9/7	1060	nil	nil	0.842	8.925
		died on 9/7/50			
urine ex bladder (30cc.)		nil	nil	1.665	0.500

Urine samples

Date	Daily excretion cc.	Vitamin B_1		Pyruvic acid mg.	
		µg/1cc.	Daily excretion mg.	per 100cc.	Daily excretion
		Sheep G			
		hay fed			
6/3/50	635	0.534	0.339	-	-
7/3	610	0.465	0.284	-	-
8/3	910	0.582	0.530	-	-
	Mean value	0.521	0.384		

Urine samples

Date	Daily excretion cc.	Vitamin B_1		Pyruvic acid mg.	
		µg/1cc.	Daily excretion mg.	per 100cc.	Daily excretion
		Sheep G - dried bracken fed			
20/3/50	920	0.216	0.199	1.684	15.493
21/3	810	0.0919	0.074	2.105	17.051
22/3	720	0.0588	0.042	3.770	27.144
23/3	437	0.0425	0.019	5.124	22.392
28/3	650	0.0328	0.021	5.435	35.327
29/3	780	nil	nil	2.452	19.126
30/3	1270	nil	nil	1.592	20.218
31/3	920	nil	nil	1.665	15.318
5/4	730	nil	nil	2.013	14.695
6/4	900	nil	nil	1.775	15.975
7/4	1020	nil	nil	1.684	17.177
8/4	1090	nil	nil	1.702	18.552
13/4	950	nil	nil	1.702	16.168
20/4	1270	nil	nil	1.592	20.218
27/4	1400	nil	nil	2.562	35.868
4/5	1550	nil	nil	2.105	32.628
11/5	1315	nil	nil	1.830	24.065
18/5	1430	nil	nil	1.556	22.251
22/5	2060	nil	nil	1.629	33.557
23/5	1675	nil	nil	1.665	27.889
24/5	1550	nil	nil	1.482	22.971
24/5 (10a.m-4p.m.)	940	nil	nil	7.046	16.890
		died on 24/5/50 at 4 p.m.			
urine ex bladder	60	nil	nil	12.517	7.510

APPENDIX 11

Hay + NaCN feeding

Vitamin B_1 and pyruvic acid in urine

Urine samples

Date	Daily excretion cc.	Vitamin B_1		Pyruvic acid mg.	
		µg/1cc.	Daily excretion mg.	per 100cc.	Daily excretion
		Bullock 5			
		hay fed			
6/3/50 (5/3 at 6 p.m.)	2800	1.760	-	-	-
7/3 (24 hours)	3125	1.942	6.069	-	-
8/3 (24 hours)	1300*	2.640	-	-	-
	Mean value	2.114	6.069		

* This collection of urine was incomplete, part being lost by accident.

Urine samples

Date	Daily excretion cc.	Vitamin B_1		Pyruvic acid mg.	
		µg/1cc.	Daily excretion mg.	per 100cc.	Daily excretion
		Bullock 5			
		Hay + NaCN fed			
20/3/50	2970	2.448	7.277	2.946	87.496
21/3	2820	2.072	5.837	3.001	84.628
22/3	2700	2.393	6.461	2.379	64.233
23/3	2810	1.834	5.154	2.452	68.901
28/3	2710	1.624	4.401	2.580	69.918
29/3	3100	1.960	6.076	3.056	94.730
30/3	2860	2.024	5.789	2.434	69.612
31/3	3070	1.904	5.845	2.507	76.965
5/4	2100	2.382	5.002	3.880	81.480
6/4	2320	2.420	5.614	3.459	80.249
7/4	2130	2.414	5.142	4.227	90.035
8/4	2310	2.338	5.401	2.946	68.053
13/4	3380	1.997	6.750	2.178	73.616
20/4	2130	1.980	4.217	4.648	99.002
27/4	2420	2.632	6.369	3.056	73.955
4/5	3120	1.764	5.504	3.642	113.630
13/5	2850	1.697	4.836	2.791	79.544
		Dried bracken alcoholic extract			
9/7	3316	1.723	5.713	2.233	74.046

Date	Urine samples: Daily excretion cc.	Vitamin B_1: µg/1cc.	Vitamin B_1: Daily excretion mg.	Pyruvic acid mg.: per 100cc.	Pyruvic acid mg.: Daily excretion
		Sheep H			
		hay fed			
6/3/50	610	1.426	0.870	-	-
7/3	650	1.332	0.866	-	-
8/3	815	1.702	1.387	-	-
20/3	750	-	-	2.141	16.057
21/3	730	-	-	2.068	15.100
22/3	695	-	-	1.830	12.719
23/3	810	-	-	1.702	13.786
	Mean value	1.487	1.041	1.935	14.416

Date	Daily excretion cc.	Vitamin B_1		Pyruvic acid mg.	
		µg/1cc.	Daily excretion mg.	Per 100cc.	Daily excretion
		Urine samples			
		Sheep H			
		Experimental control period			
		hay fed			
5/4/50	440	0.491	0.216	2.489	10.952
6/4	450	0.914	0.411	2.452	11.034
7/4	515	0.550	0.283	2.105	10.841
8/4	640	0.565	0.362	1.226	7.856
9/4	650	0.868	0.564	1.556	8.114
10/4	670	0.999	0.670	1.409	9.440
11/4	530	1.082	0.573	1.684	8.925
12/4	620	0.989	0.613	1.830	11.346
13/4	600	1.296	0.778	1.629	9.774
14/4	710	1.060	0.753	1.684	11.956
18/4	620	1.421	0.881	2.233	13.845
20/4	640	1.403	0.898	1.848	11.827
24/4	540	1.073	0.579	2.068	11.167
25/4	510	1.131	0.577	1.702	8.680
26/4	630	0.941	0.593	2.141	13.488
27/4	710	1.232	0.875	2.379	16.891
28/4	810	1.890	1.531	2.397	19.416
29/4	735	1.221	0.897	1.903	13.987
30/4	750	1.554	1.166	2.242	16.815
2/5 /					

Urine samples					
Date	Daily excretion cc.	Vitamin B_1		Pyruvic acid mg.	
		µg/1cc.	Daily excretion mg.	Per 100cc.	Daily excretion
Sheep H					
Experimental control period					
hay fed (Cont'd)					
2/5/50	790	1.593	1.259	-	-
3/5	740	1.989	1.472	-	-
4/5	700	1.934	1.354	2.361	16.527
5/5	700	1.595	1.117	-	-
6/5	720	0.944	0.680	-	-
7/5	710	-	-	-	-
8/5	580	-	-	-	-
9/5	670	1.650	1.106	-	-
10/5	710	1.566	1.112	-	-
11/5	570	1.815	1.035	2.507	14.290
Hay + NaCN dosed					
22/5	690	1.485	1.025	2.105	13.525
1/6	730	0.984	0.718	2.013	14.695
8/6	780	0.945	0.737	2.379	18.556
9/7	700	1.511	1.0577	1.702	11.914

APPENDIX 12

Blood prothrombin test

Fresh bracken - 2nd Experiment

18/8/49 - 24/10/49

Prothrombin Time (Secs.)

Date	Plasma Dilutions 5%	10%	15%	20%	50%	100%
			Bullock 4			
6/9/49	54.5	35.0	28.0	27.2	22.0	21.4
13/9	43.2	41.2	32.4	23.8	-	-
20/9	37.0	31.5	17.0	16.0	15.3	-
27/9	49.0	30.0	30.4	28.8	20.6	17.3
4/10	54.5	41.2	37.0	34.7	25.8	24.6
14/10	83.1	45.5	35.8	37.0	26.0	23.0
21/10	42.0	32.0	26.4	-	25.5	24.4
Day of death 24/10	53.2	39.8	39.2	33.2	26.6	25.0
Mean values 6/9-24/10/49	52.1	37.0	30.8	28.7	23.1	22.6
			Sheep C - normal control - hay fed			
6/9/49	153.0	69.7	45.5	36.4	32.9	31.9
13/9	140.0	54.0	47.0	44.5	33.8	30.2
27/9	111.0	56.0	56.2	54.6	50.8	32.2
Mean values	135.0	59.9	49.6	45.2	39.2	31.4

APPENDIX 12 (Cont'd)

Prothrombin time (Secs.)

Date	Plasma dilutions 5%	10%	15%	20%	50%	100%
			Sheep E			
6/9/49	104.0	37.0	32.1	27.6	7.7	5.0
13/9	51.0	37.5	40.0	31.8	24.0	21.2
27/9	63.6	44.8	37.4	33.2	26.6	24.8
4/10	79.8	70.0	42.6	35.2	31.9	33.5
14/10	72.4	48.8	-	-	27.7	24.6
Day of death 21/10	53.0	44.5	44.0	39.0	31.4	30.8
Mean values 6/9-21/10/49	70.6	47.1	39.2	33.4	24.9	23.3
			Sheep F			
6/9/49	63.2	39.0	8.1	6.8	2.0	1.0
13/9	122.0	49.0	40.6	32.0	24.8	29.0
27/9	97.5	49.0	49.6	29.2	24.8	24.7
4/10	59.2	42.6	33.0	30.8	25.4	23.9
14/10	65.0	39.0	34.8	31.4	25.6	22.8
Day of death 23/10	183.0	72.0	54.8	45.6	34.4	30.5
Mean values 6/9-23/10/49	98.3	48.4	36.8	29.3	22.8	22.0

APPENDIX 13

Blood Analyses

Fresh bracken - 2nd Experiment

18/8/49 - 24/10/49

Date	Samples	Specific Gravity		Plasma Proteins gm/100cc.	Haemoglobin gm/100cc.	Haematocrit
		Blood	Plasma			
			Bullock 4			
17/8/49	Normal (pre-experimental)	1.052	1.027	7.18	12.05	35.09
30/8	Experimental	1.052	1.0275	7.35	11.90	35.50
6/9		1.0535	1.0270	7.15	12.80	38.00
13/9		1.054	1.028	7.53	12.80	37.90
20/9		1.055	1.0295	8.06	12.80	38.00
27/9		1.053	1.0295	8.05	11.90	35.00
4/10		1.055	1.028	7.53	13.28	39.50
14/10		1.054	1.025	6.45	13.70	40.60
21/10		1.050	1.028	7.52	10.90	32.20
30/8-21/10/49	Experimental mean	1.0533	1.0278	7.46	12.51	37.10
Day of death 24/10/49		1.030	1.023	5.75	3.70	10.09

Date	Samples	Specific Gravity Blood	Specific Gravity Plasma	Plasma Proteins gm/100cc	Haemoglobin gm/100cc	Haematocri
		Sheep C - Normal control - hay fed				
17/8/49		1.044	1.022	5.38	10.03	29.5
31/8		1.045	1.023	5.73	10.10	30.0
6/9		1.046	1.023	5.72	10.64	31.5
13/9		1.045	1.024	6.10	9.95	29.0
27/9		1.045	1.022	5.38	10.50	30.9
17/8 - 27/9/49	Mean of control period	1.045	1.023	5.66	10.24	30.2
		Sheep E				
17/8/49	Normal (pre-experimental)	1.047	1.024	6.10	10.80	31.9
30/8	Experimental	1.049	1.025	6.46	11.19	33.6
6/9		1.046	1.026	6.81	9.70	28.4
13/9		1.049	1.026	6.82	11.00	32.6
27/9		1.046	1.026	6.81	9.70	28.4
4/10		1.045	1.025	6.46	9.50	27.9
14/10		1.046	1.025	6.47	10.00	29.5
30/8 - 14/10/49	Experimental mean	1.047	1.0255	6.64	10.18	30.07
Day of death 21/10/49		1.048	1.024	6.10	11.20	33.0

Date	Samples	Specific Gravity		Plasma Proteins gm/100cc	Haemoglobin gm/100cc	Haematocrit
		Blood	Plasma			
			Sheep F			
17/8/49	Normal	1.045	1.022	5.49	10.50	30.9
30/8		1.046	1.024	6.10	10.39	30.6
6/9		1.047	1.024	6.10	10.79	31.9
13/9	Experim-ental	1.046	1.024	6.10	10.39	30.6
27/9		1.0465	1.023	5.73	10.82	31.9
4/10		1.046	1.024	6.10	10.39	30.6
14/10		1.044	1.022	5.38	10.00	29.5
30/8 - 14/10/49	Experimental mean	1.046	1.024	5.92	10.46	30.85
Day of death 23/10/49		1.037	1.019	4.30	7.90	23.1

APPENDIX 14

Urine - Total benzoic acid

Fresh bracken

No.	Date	Feeding	Total benzoic acid per 100cc urine gm.	Total benzoic acid in daily excretion gm.
		1st Experiment - (18/6/49 - 17/7/49)		
		Bullock 1		
1	17-18/6 (2 days)	Hay	1.41	73.89
2	19/6-4/7 (16 days)	Bracken	1.48	82.55
3	5/7-17/7 (13 days)	"	1.81	85.46
4	18/7	Day of death	0.65	25.68
	Mean value (2 and 3) =		1.65	84.01

No.	Date	Feeding	Total benzoic acid per 100cc urine gm.	Total benzoic acid in daily excretion gm.
		2nd Experiment - (18/8/49 - 24/10/49)		
		Bullock 4		
1	13-14/8 (2 days)	Hay	1.99	24.45
2	15-16/8 (2 days)	"	1.90	29.35
3	17-18/8 (2 days)	"	1.88	26.48
		Mean value =	1.92	26.76
4	19-30/8 (12 days)	Fresh bracken	1.39	26.25
5	31/8-11/9 (12 days)	" "	1.91	24.11
6	12-23/9 (12 days)	" "	2.31	33.14
7	24/9-5/10 (12 days)	" "	2.56	34.76
8	6-9/10 (4 days)	" "	2.04	23.87
9	10-13/10 (4 days)	" "	1.97	27.14
10	14-17/10 (4 days)	" "	1.95	30.37
11	18-21/10 (4 days)	" "	1.78	27.38
12	22/10/49 (1 day)	" "	1.01	14.75
		Mean value (4 - 12) =	1.99	28.38

No.	Date	Feeding	Total benzoic acid per 100cc urine gm.	Total benzoic acid in daily excretion gm.
		Sheep C		
1	18-30/8 (13 days)	Hay	1.07	8.84
2	31/8-11/9 (12 days)	"	0.98	6.89
3	12/9-27/9 (16 days)	"	1.09	7.99
		Sheep D		
4	28/9-9/10 (12 days)	"	1.35	10.03
5	10/10-21/10 (12 days)	"	1.28	9.54
	Mean value (C and D) =		1.15	8.66
		Sheep E		
1	18/8	Hay	1.11	10.55
2	19/8-3/9 (11 days)	Fresh bracken	0.45	2.85
3	4/9-19/9 (16 days)	" "	0.54	1.33
4	20/9-5/10 (16 days)	" "	1.21	3.27
5	6/10-21/10 (16 days)	" "	0.78	4.34
	Mean value (2 - 5) =		0.75	2.95

No.	Date	Feeding	Total benzoic acid per 100cc urine gm.	Total benzoic acid in daily excretion gm.
		Sheep F		
1	18/8	Hay	0.91	11.74
2	19/8-3/9 (16 days)	Fresh bracken	0.52	6.06
3	4/9-19/9 (16 days)	" "	1.71	10.38
4	20/9-5/10 (16 days)	" "	2.43	15.65
5	6/10-23/10 (18 days)	" "	1.20	12.21
	Mean value (2 - 5) =		1.47	11.08

APPENDIX 15

Urine - Free benzoic acid

NaCN and dried bracken feeding

No.	Date	Feeding	Free benzoic acid per 100cc urine gm.	Free benzoic acid in daily excretion gm.
		Bullock 5 (15/3/50 - 21/5/50)		
1	15/3/50	Hay	0.134	3.551
2	18/3	NaCN + Hay	0.080	2.088
3	19/3	" "	0.079	2.125
4	27/3	" "	0.050	1.640
5	3/4	" "	0.042	1.088
6	12/4	" "	0.079	2.228
	Mean value (2 - 6) =		0.066	1.834
		Sheep D (15/3/50 - 12/4/50)		
I	15/3/50	Hay	0.113	0.859
2	18/3	Dried bracken	0.068	0.816
3	19/3	" "	0.067	0.717
4	27/3	" "	0.077	0.408
5	3/4	" "	0.114	0.308
6	12/4	" "	0.070	0.266
	Mean value (2- 6) =		0.079	0.503

	Date	Feeding	Free benzoic acid per 100cc urine gm.	Free benzoic acid in daily excretion gm.
		Sheep G (15/3/50 - 12/4/50)		
I	15/3/50	Hay	0.074	0.585
2	18/3	Dried bracken	0.033	0.611
3	19/3	" "	0.036	0.382
4	27/3	" "	0.097	0.514
5	3/4	" "	0.102	0.612
6	12/4	" "	0.068	0.721
		(2 - 6) Mean Value	0.067	0.568